United States Department of Agriculture

Cooperative State Research, Education, and Extension Service

Competitive Research Grants and Awards Management

NRI Annual Report: Fiscal Year 2001

National Research Initiative Competitive Grants Program

Web – *http://www.reeusda.gov/nri*
Phone – 202-401-5022
E-mail – nricgp@reeusda.gov

This annual report and other NRI materials such as *Abstracts of Funded Research*, the *NRI Program Description*, *NRI Research Highlights*, and *NRI Cover Stories* are available on the NRI home page (*http://www.reeusda.gov/nri*). For more information about the NRI, write or call the National Research Initiative Competitive Grants Program, Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture, Mail Stop 2241, 1400 Independence Ave., SW, Washington, DC 20250-2241; 202-401-5022; nricgp@reeusda.gov.

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National Research Initiative Competitive Grants Program

Cooperative State
Research, Education,
and Extension Service

Competitive Research
Grants and Awards
Management

"Knowledge for Tomorrow's Solutions"

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Message from the NRI Chief Scientist

Dear Colleagues and Interested Parties:

In FY 2001, the NRI had a successful year of investing in the future of exciting agricultural research opportunities, with funding of $105.6 million. While the FY 2001 level was a reduction of 11% below FY 2000, continuing Administration and Congressional support for the NRI is clearly indicated by the fact that the Department's FY 2002 Appropriations Act provides $120 million for the program and the President's FY 2003 budget requests $240 million.

The NRI met the FY 2001 funding constraint by retaining as many existing programs as possible, while suspending those programs where alternative avenues of support were available, either within or outside the USDA. The Ecosystems, Plant Genome, and Animal Genome programs were not offered in FY 2001 because these areas of research were covered, albeit with different emphases, through the National Science Foundation (NSF), through the USDA's Initiative for Future Agriculture and Food Systems (IFAFS) program, and in general through other NRI programs.

The NRI Plant Genetic Mechanisms program expanded its research area of support to include functional genomics, quantitative trait loci analyses, and comparative mapping. Also, in cooperation with IFAFS, we were able to offer a program in microbial genomics in a coordinated request with NSF. The effort to reorganize and suspend certain programs diminished the impact of the budget reduction on the agricultural research community as a whole, in that most NRI programs were not impacted by the reduction, and those that were suspended had alternative funding sources. The restoration of NRI funding in FY 2002 to $120 million has enabled the NRI to again offer a full complement of programs, including the ecosystems and genomics areas.

The NRI uses a time-tested competitive peer-review process to award grants supporting research in the biological, environmental, physical, and social sciences to solve regional and national problems relevant to agriculture, food, forestry, and the environment. The ultimate goal of the research is to ensure that U.S. agriculture and forestry are sustainable and globally competitive. Competition for NRI research funds is open to researchers at all U.S. academic institutions, federal research agencies, and private and industrial organizations as well as to individual researchers.

Examples of research supported by the NRI in this report show recognition of important scientific, economic, and sociological questions. A small business, dependent on prior NRI-supported research, is also highlighted. Special recognition is given to Dr. Daniel Strawn, who received a Presidential Early Career Award for Scientists and Engineers.

The NRI assessed its program portfolio to determine the broader areas in which research needs were greatest and in which the NRI contributes to the agricultural enterprise. Thus, during the year, two strategic issues were identified that would be highlighted in subsequent years. The concept for strategic issues was also recommended by the National Research Council (NRC)[1] review and others. The

[1]National Research Initiative. A Vital Competitive Grants Program in Food, Fiber, and Natural Resources Research. 2000. National Academy Press, Washington, DC, 189 pp.

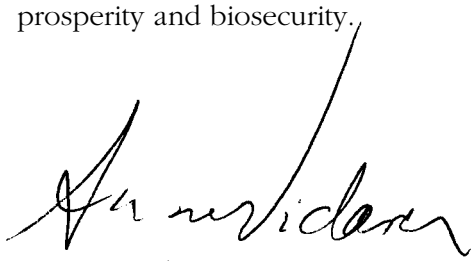
initial issues were 1) agricultural security and safety through functional genomics and 2) new and re-emerging diseases and pest threats. Several NRI programs are appropriate for these areas.

A significant research accomplishment completed at the end of 2000 and supported with NRI funding in partnership with the NSF and the Department of Energy (DOE) climaxed an international 5-year effort to determine the first complete sequence of the simple model plant *Arabidopsis thaliana*, a common roadside weed in the mustard family. *Arabidopsis* has quickly become the laboratory mouse of the plant world, studied for insights that can be applied to virtually all other plants. Its DNA code now provides scientists with a more complete genetic toolbox to engineer crops to increase food production and improve nutrition. The next steps are to determine the function of these genes for which NSF has initiated a 2010 Project. The expectation is that all gene functions can be determined by that date. The NRI also expects to participate in this endeavor of determining gene functions applicable to agriculturally significant plants.

The NRI has been remarkably successful, as determined by recognition of scientists' work, in applications to consumers and growers of food and fiber, and in use by new and established businesses. Individuals wishing to learn more about NRI-funded research can do so by reading *NRI Research Highlights*, a series of factsheets featuring successful NRI-funded research projects and their potential impact on U.S. and world agriculture, and *NRI Cover Stories*, a series of flyers depicting NRI-funded research that has been featured on the covers of prominent peer-reviewed scientific journals. Both are available on the NRI home page (*http://www.reeusda.gov/nri*).

The report that follows is an overview of some of the research the NRI will be supporting over the next 2 to 3 years from its FY 2001 appropriation. I have chosen to highlight just a few of the 597 grants funded. I have selected ones that seemed to have broad appeal and to exemplify USDA missions.

As Chief Scientist, it is an honor and privilege to be associated with such an exemplary program. I am impressed with the very capable and dedicated staff, which despite very small numbers does a superb job in administration. I also thank those who support competitive research and the many fine scientists who contribute to the mission of the NRI through their participation in the peer-review process. Competitive research in agriculture is essential for our country's future economic prosperity and biosecurity.



Anne K. Vidaver
NRI Chief Scientist

The National Research Initiative: Overview

USDA's National Research Initiative was established in 1991 in response to recommendations outlined in *Investing in Research: A Proposal to Strengthen the Agricultural, Food, and Environmental System,* a 1989 report by the National Research Council's (NRC) Board on Agriculture. This publication called for increased funding of high-priority research, funded by USDA through a competitive peer-review process, directed at:

- Increasing the competitiveness of U.S. agriculture.
- Improving human health and well-being through an abundant, safe, and high-quality food supply.
- Sustaining the quality and productivity of the natural resources upon which agriculture depends.

Continued interest in and support of the NRI is reflected in two subsequent NRC reports, *Investing in the National Research Initiative: An Update of the Competitive Grants Program of the U.S. Department of Agriculture,* published in 1994, and *National Research Initiative: A Vital Competitive Grants Program in Food, Fiber, and Natural-Resources Research,* published in 2000.

Competitive Review Process

The NRI competitive review process encourages innovative ideas that are likely to open fundamentally new research approaches to enhancing agriculture, food, forestry, and the environment. A proven mechanism for stimulating new scientific research, the process increases the likelihood that investigations addressing important, relevant topics using well-designed and well-organized experimental plans will be funded. Each year, panels of scientific peers meet to evaluate and recommend proposals based on scientific merit, investigator qualifications, and relevance of the proposed research to U.S. agriculture.

At least 10 percent of NRI funds support Agricultural Research Enhancement Awards. These awards enhance the U.S. agricultural research system through funding of postdoctoral fellowships and research by new investigators as well as through Strengthening Awards.

Strengthening Awards include Research Career Enhancement Awards, Equipment Grants, Seed Grants, and Strengthening Standard Research Projects. These grants fund researchers at small and mid-sized institutions with limited institutional funding success or in states and other entities that are part of the Experimental Program for Stimulating Competitive Research (EPSCoR).

The NRI encourages multi-disciplinary research, which is needed to solve complex problems, and seeks to initiate research in new areas of science and engineering that are relevant to agriculture, food, forestry, and the environment. The NRI also supports scientific conferences to facilitate the exchange of information necessary to achieve the most rapid advances in these areas. Both mission-linked research and fundamental research are supported by the NRI. Mission-linked research targets specific problems, needs, or opportunities. Fundamental research – the quest for new knowledge about agriculturally important organisms, processes, systems, or products – opens new directions for mission-linked research. Both mission-linked research and fundamental research are essential to the sustainability of agriculture.

Policy

Currently, a Board of Directors, chaired by the USDA Under Secretary for Research, Education, and Economics (REE), provides oversight of NRI policy. Board members include the Administrators of the four agencies comprising the REE Mission Area – the Cooperative State Research, Education, and Extension Service (CSREES); the Agricultural Research Service (ARS); the Economic Research Service (ERS); and the National Agricultural Statistics Service (NASS) – as well as the Deputy Chief for Research of the Forest Service (FS) and the NRI Chief Scientist. The Deputy Administrator of CSREES' Competitive Research Grants and Awards Management Division serves as the Board's Executive Officer.

The Board of Directors oversees NRI policy by providing comments to the CSREES Administrator on the annual *NRI Program Description,* considering the recommendations made by the National Agricultural Research, Extension, Education, and Economics Advisory Board; identifying issues of importance to the NRI; providing a forum on future directions of the NRI; and fostering communication across relevant USDA research agencies regarding NRI programs and procedures.

Under consideration is a new mechanism for providing policy and oversight. The NRC report (cited above) recommended the establishment of an extramural advisory board representing NRI stakeholders. The board would provide guidance on scientific and technological priorities, provide a forum for stakeholder concerns, assist in evaluating research outcomes, evaluate NRI operations, and provide research advocacy for peer-reviewed research within and external to USDA. Establishment of a small advisory or consultative board is under consideration.

Identification of Research Priorities

Setting research priorities is an important means of facilitating the scientific and technological advances needed to meet the challenges facing U.S. agriculture. Congress sets the basic budgetary framework for the programs of the NRI by providing funds in six priority categories (see section on Authorization, below). Members of Congress also make recommendations for the scientific and programmatic administration of the NRI through appropriation language and through their questions and comments during Congressional hearings.

Input into the priority-setting process is sought from a variety of NRI customers and stakeholders. The scientific community provides direction for the NRI through the research proposals it submits each year as well as through the research proposal evaluations and funding recommendations of individual scientific peer-review panels.

NRI scientific staff members play an important role in providing continuity of programmatic and scientific administration from year to year. Staff members attend scientific and professional meetings to stay current on scientific trends that need to be reflected in the *NRI Program Description* and in the coordination of priority setting with other federal agencies. NRI staff also participate in meetings with representatives of key commodity groups and other user groups to discuss these stakeholders' current research priorities, learn ways the NRI can assist in meeting their needs, and solicit comments and suggestions on NRI research priorities.

Input from several coalitions has proved to be an important source of information. NRI staff members meet with groups such as the Institute of Food Technologists, CoFARM, C-FARE, FAIR 2002, and the Animal Agriculture Coalition to gain a broad perspective on current research needs and priorities.

The NRI Chief Scientist, the Deputy Administrator of the Competitive Research Grants and Awards Management unit, and NRI scientific staff are responsible for assimilating the input of diverse stakeholder groups into a program description that will solicit the highest quality proposals to meet the needs of U.S. agriculture. The NRI research areas, which are evaluated and updated each year, are in the *NRI Program Description* issued annually.

The *NRI Program Description* is accessible to universities, federal research laboratories, private research organizations, and individual scientists – both in printed form and on the Internet via the NRI home page (*http://www.reeusda.gov/nri/*). In addition, the NRI receives comments on its programs from academic administrators, other staff members, and scientists from universities; the Experiment Station Committee on Organization and Policy (ESCOP); and the research administrators of the 1890 land-grant institutions.

Authorization

In the legislation that authorized the establishment of the NRI, Congress defines high-priority research as basic and applied research that focuses on both national and regional research needs (and methods for technology transfer) in the following areas:

- Plant Systems
- Animal Systems
- Nutrition, Food Quality, and Health
- Natural Resources and the Environment
- Engineering, New Products, and Processes
- Markets, Trade, and Policy

The authorizing legislation requires that, as appropriate, grants be consistent with the development of systems of sustainable agriculture. Congress further has specified that no less than 30 percent of funds be used to support multi-disciplinary team research, no less than 40 percent be used for mission-linked

research, and no less than 10 percent be used to strengthen the research capacity of individuals and institutions.

Program Implementation

The *NRI Program Description* is distributed widely within the scientific community and among other interested groups. The fiscal year (FY) 2001 *NRI Program Description and Guidelines for Proposal Preparation,* published in the *Federal Register,* identified 23 research programs within the following 8 major research areas:

- Natural Resources and the Environment
- Nutrition, Food Safety, and Health
- Animals
- Biology and Management of Pest and Beneficial Organisms
- Plants
- Markets, Trade, and Rural Development
- Enhancing Value and Use of Agricultural and Forest Products
- Agricultural Systems Research

A total of 2,593 research proposals were considered for funding in FY 2001. Twenty-six peer panels reviewed and ranked the proposals, evaluating them on scientific merit, the qualifications of proposed project personnel, the adequacy of the proposed facilities, and the relevance of the proposed project to long-range improvements in – and the sustainability of – U.S. agriculture.

Each peer panel was composed of individuals with the expertise required to review each proposal thoroughly and fairly. Proposals for Postdoctoral Fellowships, New Investigator Awards, and Strengthening Standard Research Projects were reviewed within the specified research program area. Proposals for Research Career Enhancement Awards, Equipment Grants, and Seed Grants were reviewed as a group.

Criteria for the selection of panel members included knowledge of the relevant scientific discipline, educational background, experience, and professional stature within the scientific community. The membership of each panel was carefully balanced to reflect diversity in geographical region, type of institution, type of position, and gender and minority status (see Table 1).

Table 1. Characteristics of NRI Peer Panels, FY 2001

Geographic Region	Number[1]	Percentage
North Central	68	20.5
Northeast	92	27.8
South	92	27.8
West	79	23.9
Type of Institution	**Number**	**Percentage**
Land-Grant	214	64.7
Public/Private	55	16.6
Federal	40	12.1
Industry/Other	22	6.6
Type of Position	**Number**	**Percentage**
Assistant Professor	66	19.9
Associate Professor	110	33.2
Professor	81	24.5
Federal	40	12.1
Industry	20	6.1
Other	14	4.2
Gender/Minority	**Number**	**Percentage**
Non-minority Males	199	60.1
Non-minority Females	80	24.2
Minority Males	37	11.2
Minority Females	15	4.5

[1] Reviewers in each category.

Additional expertise was brought to proposal evaluation by a number of scientists and other experts representing a wide variety of fields, who conducted *ad hoc* reviews. These reviews provided the additional expertise that made it possible to select the highest quality, most meritorious proposals for funding.

More than 9,000 scientists contributed their time and expertise to the NRI proposal evaluation process in FY 2001. Participation in the panels and in writing *ad hoc* reviews provided many individuals the opportunity to gain experience in the review process and to become more familiar with the nature of the science supported by the NRI. The pool of *ad hoc* reviewers also provided a resource from which future panel members may be selected.

At the conclusion of the review process, a summary of the panel evaluation and the written reviews were forwarded to the submitting investigators, providing them with critical assessments of their proposed research by recognized leaders in the appropriate fields. The reviewers' comments and suggestions also were important for purposes of refining the proposals for future resubmission.

Continuing a practice begun in 1993, nontechnical summaries describing each research project funded in FY 2001 will be published as *Abstracts of Funded Research* and submitted to the House and Senate Agriculture Appropriations Committees. This publication is also available via the Internet on the NRI home page (*http://www.reeusda.gov/nri/*).

Grantsmanship Workshops

NRI program staff conducted a number of workshops in FY 2001 to increase applicants' and administrators' understanding of the philosophy, directives, and procedures of the NRI competitive review process. In FY 2001, staff held a well-attended grant-writing workshop in Denver, CO, as part of its ongoing practice of conducting a major grant-writing workshop annually in one of the four U.S. regions (North Central, Northeast, South, and West). The Denver workshop was hosted by Colorado State University. It focused on factors contributing to successful proposals, guidelines for preparing proposals, individual program descriptions, and recent funding statistics. Previous panel managers were part of the program.

In addition, the NRI staff conducted individualized workshops or made presentations at national meetings of scientific and/or professional societies, for regional research groups, and other audiences, including the Nutrition, Food Safety and Health Extension Conference, Society for Nutrition Education, International Food Protection Association, Conference of Research Workers in Animal Disease, and American Veterinary Medical Association. Other workshop sites included EPSCoR institutions; 1890 land-grant institutions, including Tuskegee University; Hispanic-serving institutions, such as Texas A&M, Kingsville; and tribal colleges, such as those in Albuquerque, NM.

Funded Research

In FY 2001, a total of 2,593 proposals were submitted to the NRI, requesting a total of $645,780,324. Awards totaling $97,411,934 were made to the highest ranked 597 proposals (see Table 2).

The success rate (in terms of number of proposals funded and excluding conferences, supplements, and continuing increments of the same grant) was 23.4 percent, which is 3.3 percent higher than in FY 2000. The average grant award for new standard research projects (excluding Research Career Enhancement Awards, Equipment Grants, Seed Grants, conferences, continuing increments, and supplements) in FY 2001 was $188,116 for 2.4 years. (For FY 2000, the comparable figures were $180,473 for 2.4 years.)

The NRI provided funds totaling $588,670 in partial support of 68 conferences in FY 2001. These conferences brought scientists together to identify research needs, update one another on research information, and/or advance an area of research important to U.S. agriculture.

In FY 2001, the NRI provided funds totaling $17,165,326 in Agricultural Research Enhancement Awards. This support included Postdoctoral Fellowships, New Investigator Awards, and Strengthening Awards (see Table 3).

Crosscutting Areas

A number of research topics of major importance to USDA involve several research areas or programs. NRI support for these crosscutting program areas in FY 2001 is indicated in Table 4.

The data show the total amount of funding from all research areas for a specified research topic. For example, the Water Quality area includes projects from the Watershed Processes and Water Resources Program, as well as projects from other programs relevant to water quality such as Soils and Soil Biology. The Integrated Pest Management area includes projects funded from the programs on Biologically Based Pest Management, Entomology and Nematology, Biology of Plant-Microbe Associations, and Biology of Weedy and Invasive Plants. The $6.7 million funding allocation for sustainable

Table 2. NRI Funding Allocations[1], FY 2001

Research Area/Program	Number of Grants	Total Dollars Awarded[2]
Natural Resources & Environment		
Plant Responses to the Environment	18	$3,090,000
Watershed Processes and Water Resources	18	4,124,049
Soils and Soil Biology	23	4,380,600
Total: Natural Resources and Environment	**59**	**$11,594,649**
Nutrition, Food Safety, & Health		
Improving Human Nutrition for Optimal Health	22	4,774,458
Food Safety	31	5,976,378
Epidemiological Approaches to Food Safety[3]	6	5,486,155
Total: Nutrition, Food Safety, & Health	**59**	**$16,236,991**
Animals		
Animal Reproduction	22	3,888,726
Animal Health and Well-Being[4]	55	10,988,912
Animal Genome and Genetic Mechanisms	15	2,700,000
Animal Growth, Development, and Nutrient Utilization[5]	26	3,928,146
Total: Animals	**118**	**$21,505,784**
Biology and Management of Pest and Beneficial Organisms		
Entomology and Nematology[6]	33	5,593,143
Biology of Plant-Microbe Associations[7]	26	5,349,010
Biologically Based Pest Management[8]	17	2,494,593
Biology of Weedy and Invasive Plants	11	2,630,000
Total: Pest Biology and Management	**87**	**$16,066,746**
Plants		
Plant Genetic Mechanisms[9]	34	4,833,571
Plant Growth and Development[10]	28	3,646,000
Agricultural Plant Biochemistry[11]	34	3,914,847
Total: Plants	**96**	**$12,394,418**
Markets, Trade, & Rural Development		
Markets and Trade[12]	21	1,984,000
Rural Development[13]	14	1,483,333
Total: Markets, Trade, & Rural Development	**35**	**$3,467,333**
Enhancing Value and Use of Agricultural and Forest Products		
Food Characterization/Process/Product Research	21	3,367,883
Non-Food Characterization/Process/Product Research	16	2,453,217
Improved Utilization of Wood and Wood Fiber[14]	16	2,025,166
Total: Enhancing Value & Use of Agricultural & Forest Products	**53**	**$7,846,266**
Other		
Agricultural Systems	11	2,535,289
Strengthening Programs[15]	75	3,514,458
Metabolic Engineering Program - Interagency[16]	2	250,000
U.S. Rice Genome Project - Interagency	2	2,000,000
Total: Other	**90**	**$8,299,747**
Awards to be Determined[17]		**$1,620,267**
GRAND TOTAL	**597**	**$99,032,201**

[1]The content of this table varies slightly from tables provided in documents supporting the President's budget to Congress each year in the following ways: 1) while the documents supporting the President's budget include data only for funds from the 2001 appropriation, this table includes data on all awards from proposals submitted to the 2001 proposal cycle, regardless of the year the funds were appropriated (as noted in the table) and 2) awards are arranged in this table under program area (to which proposals are submitted and reviewed) as opposed to relationship to appropriated budgetary lines.
[2]Includes awards still in process.
[3]Includes $304,672 in funds from the FY 2000 appropriation.
[4]Includes $13,239 in funds from the FY 1996 appropriation, $6,759 in funds from the FY 1997 appropriation, and $2 in funds from the FY 1999 appropriation.
[5]Includes $1,000 in funds from the FY 1996 appropriation and $5,000 in funds from the FY 2000 appropriation.
[6]Includes $2,132 in funds from the FY 1996 appropriation, $21,981 in funds from the FY 1997 appropriation, and $4,030 in funds from the FY 1998 appropriation.
[7]Includes $30,000 in funds from the FY 1997 appropriation and $479,010 in funds from the FY 2000 appropriation.
[8]Includes $3,974 in funds from the FY 1997 appropriation.
[9]Includes $10,864 in funds from the FY 1996 appropriation, $498 in funds from the FY 1997 appropriation, $2,638 from the 1998 appropriation, and $114,571 in funds from the FY 2000 appropriation.
[10]Includes $5,000 in funds from the 1997 appropriation.
[11]Includes $2,298 in funds from the 1997 appropriation and $549 in funds from the FY 1998 appropriation.
[12]Includes $2,293 in funds from the 1996 appropriation, $5,596 in funds from the FY 1997 appropriation, $1 in funds from the FY 1998 appropriation, and $2,110 in funds from the FY 1999 appropriation.
[13]Includes $12,666 in funds from the FY 1996 appropriation, $47 in funds from the FY 1997 appropriation, $21 in funds from the FY 1998 appropriation and $2,646 in funds from the FY 1999 appropriation.
[14]Includes $4,308 in funds from the FY 1996 appropriation and $37,666 in funds from the FY 1998 appropriation.
[15]Includes $88,187 in funds from the FY 1996 appropriation, $41,133 in funds from the FY 1997 appropriation, $54,702 from the FY 1998 appropriation, and $6 from the FY 1999 appropriation.
[16]Includes $100,000 in funds from the FY 2000 appropriation.
[17]As of December 17, 2001.

agriculture represents projects identified from many NRI programs, including the Agricultural Systems Research Program, that are directly relevant to sustainable agriculture. This figure is probably an underestimate since, in a broad sense, all research supported by the NRI is germane to sustainable agriculture.

Table 3. Agricultural Research Enhancement Awards, FY 2001

Type of Award	Number of Grants	Total Dollars Awarded[1]
Postdoctoral Fellowships	18	$1,799,807
New Investigator Awards	29	4,303,499
Strengthening Awards		
Research Career Enhancement Awards	6	348,515
Equipment Grants	37	903,177
Seed Grants	32	2,262,766
Standard Strengthening Research Projects	50	7,547,562

[1]As of November 15, 2001. Includes awards still in process.

Table 4. Crosscutting Program Areas, FY 2001

Research Topic	Number of Grants	Total Dollars Awarded[1]
Plant Genome	102	$10,317,106
Forest Biology	19	2,799,653
Global Change	26	3,794,959
Sustainable Agriculture	61	12,027,721
Animal Genome	79	10,782,107
Animal Health	70	15,794,438
Water Quality	27	5,035,088
Food Safety	67	14,603,472
Integrated Pest Management	56	8,617,088

[1]As of November 15, 2001. Includes awards still in process.

Research Dimensions

As noted earlier, research programs can be examined from perspectives such as type of investigation (fundamental or mission-linked) and organization of research approach (single discipline or multi-disciplinary).

- The NRI defines *fundamental research* as that which tests scientific hypotheses and provides basic knowledge that allows advances in applied research and from which major conceptual breakthroughs are expected to occur.
- In contrast, *mission-linked research* is that which focuses on specifically identified agricultural problems, which, through a continuum of efforts, provides information, and technology that may be transferred to users and may relate to a product, practice, or process.
- *Multi-disciplinary research* is defined as work on which investigators from two or more disciplines are collaborating closely. These collaborations, where appropriate, may integrate the biological, physical, chemical, or social sciences.

NRI funding in FY 2001 for these categories is shown in Table 5.

Table 5. Dimensions of NRI Research, FY 2001

Dimension	Amount of Support	Percent
Fundamental	$44,198,983	45.4
Mission-linked	53,212,951	54.6
Multidisciplinary	35,978,046	36.9
Single discipline	61,433,889	63.1

[1]As of November 15, 2001. Includes awards still in process.

Intra-Agency Research

NRI program directors work closely within USDA to complement programs where possible and maximize intra-agency cooperation. An example of such cooperation is the barley consortium. In the United States, barley and wheat (members of the *Triticeae* family) are sown on about 80 million acres annually, with an average value of $9 billion. In 1999, the NRI funded a barley consortium to sequence 50,000 expressed sequence tags (ESTs) and anchor 600 of them to the physical map. In the past 3 years, joint projects among the investigators involved have successfully transferred their discoveries through 52 peer-reviewed and invited publications, 23 invitations to present seminars at universities and at national and international meetings, and 50 submissions of gene sequences to the national data bank, GenBank.

In addition, more than 50,000 ESTs have been submitted from the NRI-funded barley EST project, in conjunction with additional IFAFS (Initiative for Future Agriculture and Food Systems) funding to enable the consortium to develop a publicly available expression library based on a unique chip or barley microarray. Data from the microarray analysis may enable determination of abiotic and biotic stresses, such as those associated with drought and diseases that reduce crop productivity, and further integrate markers to genetic and physical maps for breeders to use for wheat and barley improvement.

Interagency Research

NRI program directors work closely with their research-funding counterparts in other federal agencies to avoid duplication and maximize interagency cooperation. Each collaborative research program issues a single request for proposals, and agency representatives work together to assemble a panel of scientific peers to identify the most meritorious proposals. From this group, representatives of each agency select proposals that are the most germane to the mission of that agency. Examples of cooperation in research that NRI funds jointly with other federal agencies include:

- The Interagency Metabolic Engineering Program, established in 1998 with the Department of Energy (DOE), the National Science Foundation (NSF), the Department of Commerce (DOC), the Department of Defense (DOD), the Environmental Protection Agency (EPA), and the National Aeronautics and Space Administration (NASA).
- The International Rice Genome Sequencing Project (IRGSP), begun in 1999, is a worldwide consortium of scientists, with the goal of obtaining the complete sequence of the rice genome. The NRI, in partnership with NSF and DOE, is providing funding for U.S. groups to participate in this activity. The IRGSP is sequencing the same strain of rice, shares materials, and releases sequences to public databases as soon as they are obtained. In February 2001, the IRGSP agreed to speed its sequencing rate by releasing high-quality draft sequence, while at the same time continuing to obtain finished sequence. Currently, the IRGSP has published nearly half of the genome sequence, and it has a goal of completing sequencing for the entire genome by the end of 2002. Initial gene number predictions indicate that the average rice gene is a little more than 2,000 bases in length; at least half of these genes have, as yet, no predicted function. Thus, a challenge for the future will be to improve computational methods for gene prediction in large, complex genomes such as rice.

The National Research Initiative: Accomplishments and Promising Research

In FY 2001, the NRI funded 597 grant proposals. This section provides examples of fundamental and mission-linked research targeted at problems important to the USDA mission, funded through the 26 panels and related to the five broad outcomes outlined in CSREES' *Government Performance and Results Act Strategic Plan*.

Outcome 1: An agricultural production system that is highly competitive in the global economy

Resistance to Horn Fly Feeding by Targeting Salivary Factors. The economic impact on livestock production in North America by the blood-sucking fly *Haematobia irritans* (horn fly) is estimated at almost $1 billion a year. Control of this insect is difficult because of its widespread resistance to insecticides. In the past 5 years, through NRI funding, **Drs. Eddie** and **Mary Cupp** of **Auburn University** have characterized the major chemicals in horn fly saliva that help these insects obtain blood. They discovered and purified a protein, thrombostasin (TS), that delays blood clotting and have cloned the genetic information (cDNA) encoding this factor. The cDNA was used to produce large quantities of a recombinant form (rTS). Having this material will now enable them to determine if blood feeding by horn flies can be affected by immunizing rabbits to generate a specific immune response. During the next year, they will test the idea that differences in the molecular structure of TS may result in differential feeding success of horn flies. By studying this important aspect, it should be possible to design an anti-feeding vaccine using the major form(s) of TS that are most effective in stimulating an immune response.

Genetics of Heliothine Resistance to Bacillus thuringiensis and Its Toxins. Some insects have become virtually immune to most insecticides. This poses a major problem for society because it typically leads to farmers using mixtures of highly toxic compounds to combat these insects. Over the years some farmers have tried to protect their crops using a natural insecticide produced by the bacterium *Bacillus thuringiensis*. This insecticide, called *Bt*, is considered to be environmentally benign because it is toxic to a few important pest species, but has no effect on almost all other insects, or on any vertebrates. Recently, genetic engineers moved the genes for producing *Bt* insecticides from the bacterium into the chromosomes of crop plants. The *Bt* toxins are now produced by cotton and corn plants on more than 20 million acres of land. The toxin is produced from the seedling stage until harvest, so there is significant and constant selection for pest evolution of resistance to the *Bt* toxins. Such resistance could cause farmers to return to the use of less benign and more expensive insecticides.

Dr. Fred Gould at **North Carolina State University** works with a number of strains of two major pest species that have become resistant to *Bt* based on selection with *Bt* in the laboratory. His team will determine which genes lead to *Bt* resistance and how these genes interact with each other to produce high levels of resistance. They will use molecular mapping techniques to localize these resistance genes. Once we understand how these genes operate, we will be better able to develop accurate ways to interfere with evolution of resistance in the field.

Dr. Gould said, "My research program has benefited dramatically from NRI financial support, and it has also benefited directly from the NRI peer-review process. Without the NRI, I would not have a research program."

Effects of a Novel Bacterium Associated with Parthenogenesis in Encarsia. The symbiotic bacterium *Wolbachia* has diverse reproductive effects on its insect hosts, including parthenogenesis (females producing daughters without mating) in species with haplodiploid genetic systems (in which females develop from diploid eggs and males from haploid eggs). The bacterium causes the chromosome complement of an incipient male egg to double and develop as a female. *Wolbachia* has been thought to be unique in its ability to cause parthenogenesis. *Wolbachia* and other symbiotic bacteria are likely to be an unacknowledged factor in many pest management programs. In addition to the potential liabilities of infection, these bacterial agents may also have tremendous potential as tools to manipulate populations in beneficial ways.

Dr. Martha Hunter at the **University of Arizona, Tucson** recently found a previously undescribed bacterium that is vertically transmitted, unrelated to

Wolbachia, and appears to cause parthenogenetic reproduction in *Encarsia*, an economically important genus of parasitoid wasps that attacks whitefly and armored scale pests. Dr. Hunter proposes to determine the effects of this newly discovered bacterium in *Encarsia* spp. hosts. The understanding we gain in studying this new bacterium will broaden our general understanding of the means by which *Wolbachia* and other bacteria manipulate their hosts and will give insight into how to best use them to improve pest management.

Altering Sex Ratio at Birth in Livestock. It is often beneficial for livestock producers to have a preponderance of offspring that are of one sex. In the dairy industry, for example, female calves are preferred because they provide future replacement heifers for the milking herd. Moreover, the dairy industry currently faces a critical shortage of available replacement heifers. In contrast, beef and pork producers often prefer male offspring because males grow more rapidly and/or have greater efficiency of feed conversion to edible meat.

Dr. R. Michael Roberts, **University of Missouri**, has determined that the development of female bovine embryos is compromised relative to males as the concentration of glucose present in the culture medium is increased. These data may help explain why dairy cows in excellent body condition tend to produce more male than female calves. Members of the laboratory have also determined that female embryos produce greater amounts of a signaling protein (called interferon-tau) than male embryos, which may influence pregnancy. These initial observations will be investigated further to determine if practical procedures can be developed to modulate the sex ratio at birth and to discover the basis for the developmental differences between the sexes before the gonads form.

Developing Alternative Methods of Sterilization for Livestock. The primary method for preventing reproduction in domestic livestock in the U.S. today is by surgical removal of the testes or ovaries; this eliminates both fertility and sexual behavior. Although the procedure is routine in males, it is not without problems – including infection and a retarded growth rate for several days following the surgery. Unfortunately, the practical usefulness of this procedure is limited to males due to inaccessibility of the female gonads. However, it is sometimes equally desirable to eliminate fertility and sexual behavior in females. For example, 15 percent of beef heifers entering feedlots are pregnant. These unintended pregnancies result in reduced efficiency of feed utilization, complications arising from pregnancy in market cattle, and ultimately, significant economic loss to the beef industry.

Dr. Terry Nett, **Colorado State University**, is undertaking research to develop an injectable formulation that will permanently sterilize either sex of a variety of economically important species. His approach is to take advantage of the toxicity of a naturally occurring plant protein (called pokeweed antiviral protein or PAP) by attaching it to gonadotropin-releasing hormone (GnRH) that is responsible for controlling reproduction in both males and females of all mammals and birds. Injection of this GnRH-PAP complex into an animal is expected to cause death of the cells responsible for stimulating the testes or ovaries without harming other cells in the body. Development of this type of technology could yield safe, convenient, and inexpensive methods for producers to sterilize livestock, when desirable, while also ameliorating animal welfare concerns about the current practice of surgical castration.

Determining Causes of Male Infertility in Poultry. A major component of the success of the broiler (meat chicken) industry has been the great improvement in growth rate that has been achieved through genetic selection and resulting production efficiency. However, intensive selection for growth has been accompanied by a progressive reduction in reproductive performance of the parental stock, to the extent that reduced male fertility may become a limiting factor to further improvement in productivity. Despite the importance of the problem, the precise causes underlying male fertility reduction have not yet been determined.

Dr. Inmaculada Estevez, **University of Maryland**, is attempting to determine the fundamental behavioral and morphological causes of reduced fertility in broiler breeder strains that have been selected for high yield. This project is unique in that it applies new concepts from behavioral theory to the improvement of fertility. Her research has a multidisciplinary approach that will allow her and

her research team to determine the complex relationships between male social and reproductive behavior and its subsequent consequences at the physiological level. The research includes determination of male phenotypes that can be used to indicate male reproductive potential and experiments conducted under commercial conditions in which the impact of the new technology will be evaluated. The goal of these studies is to develop and eventually implement approaches that may reverse the decline in reproductive efficiency that has occurred along with increased growth rates.

The Role of Mitochondrial DNA in Growth and Nutrient Utilization. Studies funded by the NRI will investigate how variations in mitochondrial DNA (a small DNA passed to offspring exclusively from the mother) influence growth and nutrient utilization. Mitochondrial DNA are within the mitochondria, sub-cellular structures important in energy metabolism. Effects of mitochondrial DNA variation are poorly understood in animals used to produce food and fiber but may include effects on growth rate and the efficiency of feed conversion to muscle.

Using a procedure called androgenesis, **Dr. Gary Thorgaard**, **Washington State University**, will examine the role of mitochondrial DNA in growth of rainbow trout. Androgenesis is a method of producing fish offspring that have inherited all of their nuclear genes from the male parent. This makes it possible to produce clonal lines in rainbow trout and other fishes. These "clones" are identical in their nuclear DNA but can be manipulated to contain different mitochondrial types. This provides an opportunity to study the effects of mitochondrial DNA independently of differences in nuclear genes.

Dr. Walter Bottje, **University of Arkansas**, is studying how differences in mitochondrial proteins that are encoded for by mitochondrial DNA contribute to variations in feed conversion and growth rate in broilers (meat chickens). He and his research team have obtained evidence that mitochondria may be involved in how efficiently broilers grow. In addition to elucidating some of the biological mechanisms underlying animal growth, this research may contribute to development of selective breeding programs for important economic traits such as growth rate and feed-to-gain ratio.

Nutritional Genomics of Dairy Cattle Will Identify Genes Regulating Milk Production and Animal Health. The transitional period from 3 weeks before to 3 weeks after calving (the peripartal period) is the most critical phase of the lactation cycle for dairy cows. Suboptimal nutrition, management, or environment during this time may limit peak milk production. The occurrence of health problems is centered disproportionately on this short period. Regulation of metabolism, health, and productivity in response to nutrition during this period is poorly understood.

Dr. James Drackley and colleagues, **University of Illinois**, will determine how dietary manipulation during the peripartal period in dairy cows influences the pattern of expression of genes that regulate metabolism, health, feed intake, and milk production. Using DNA microarray technology, the investigators expect to identify genes that are differentially regulated during both optimal and turbulent transitions from late gestation to early lactation. Results from this research in nutritional genomics will provide a basis for nutrition and management recommendations to optimize production and health of dairy cattle during the peripartal period.

Poultry Diseases. Marek's disease virus (MDV), a herpesvirus, causes heavy losses in the poultry industry annually. While vaccines are available, highly virulent strains of MDV have been isolated over the past 5 years that are able to break through the induced immunity. Recently, **Dr. Hsien-Jen Kung** at the **University of California-Davis** discovered a new gene of MDV that contributes to its pathogenic capabilities. The protein that is encoded by the gene mimics a cellular protein that recruits immune cells. This allows MDV to attract the cells that mount the immune response, infect, and inactivate them. Concurrently, **Dr. K. Anton Schat** at **Cornell University** found viral proteins that are differentially targeted by chicken immune cells. Immune cells in susceptible chicken lines do not recognize the proteins as foreign while the immune cells of resistant lines do. Therefore, it would appear that multiple factors need to be considered in MDV control.

A group of researchers under the direction of **Dr. Moses Njenga** at the **University of Minnesota** has chosen a multi-disciplinary approach to control avian pneumovirus (APV), a newly emergent pathogen of turkeys. The approach includes looking at the genetics of both the virus and the turkey. Recent studies showed that the APV strains in the U.S. are genetically and antigenically different from the European strains. This demonstrates the need for separate vaccines for the U.S. strains. One part of the project, therefore, is to develop a reverse genetics system to generate candidate vaccine viruses. The second part of the project, under the direction of **Dr. Vivek Kapur**, of the same institution, deals with the immunogenetics of the turkey to understand the molecular basis that controls susceptibility or resistance. Taken as a whole, the project should increase our knowledge of host-pathogen interactions.

Better Bacteria for Fuel Production from Agricultural Wastes. In the U.S., cornstarch contributes about 2 billion gallons of ethanol each year, which is more than 1 percent of the total automotive fuel. Although the supply of grain-derived ethanol can increase, competing markets for food would prevent expansion to the levels required to replace imported petroleum for fuel. The production of inexpensive sugar from lignocellulosic (straw) agricultural residues is the key to replacing imported petroleum. Sugars derived from renewable plant residues can serve as alternative feedstock to produce fuel ethanol, bulk chemicals, and biodegradable plastics. **Dr. Lonnie Ingram** at the **University of Florida** has been genetically engineering bacteria for fuel ethanol production. His research focuses on the development of bacteria that produce a portion of the enzymes for the solubilization of cellulose. These enhanced strains may decrease the cost of the cellulose hydrolysis process used for ethanol production.

Closterovirus and Insect Interactions. Citrus Tristeza Virus (CTV) causes the most devastating virus disease of citrus, has destroyed entire citrus industries throughout the world and threatens those throughout the U.S. The efficient aphid vector (the brown citrus aphid) entered Florida in 1995 and is killing 20 percent of the Florida trees on the susceptible sour orange rootstock. The Texas and California industries are similarly threatened. Development of resistant trees is the most desirable option. However, approximately 20 years is required to produce virus-resistant trees for use in the field. The interim management procedure is to genetically engineer mild strains that will interfere with superinfection by severe isolates, as proposed by **Dr. William Dawson, University of Florida, Citrus Research and Education Center, Lake Alfred, FL**.

One requirement for cross-protecting strains is that they must lack the ability to be transmitted by insects to other citrus varieties that might be susceptible to the cross-protecting virus. To engineer such isolates includes understanding virus and insect interactions. This requires that we understand how virions are assembled and what viral gene products are needed for aphid transmission. In this project, the investigator will examine the formation of virion complexes that are specifically acquired and transmitted by aphids and create mutants and hybrids to define viral genes required for aphid transmission. These studies may enable development of management strategies for CTV-induced diseases in a sustainable approach to retain a viable American citrus industry.

Role of Polyunsaturated Fatty Acids in the Aspergillus and Seed Interaction. Grain and legume seed are attacked by relatively few fungal genera. However, the fungi that do colonize seed, most commonly members of the genera *Aspergillus*, *Fusarium* and *Penicillium,* cause tremendous yield loss through tissue destruction as well as a significant health problem by the production of mycotoxins in the seed. The mycotoxigenic fungi are facultative pathogens that form intimate associations with grain and legume seeds. Under certain environmental conditions, the fungi will produce copious amounts of toxic, teratogenic, and carcinogenic toxins in these living seeds. At other times the fungi will colonize the seed but not produce mycotoxins. These diseases, considered some of the most serious and challenging agricultural problems, result in the most yield loss of any plant disease type – well into billion-dollar losses every year. Aside from the yield loss, there are also health effects in humans and animals that eat contaminated food.

Dr. Nancy Keller and colleagues at the **University of Wisconsin-Madison** have provided evidence

that there is a sophisticated lipid-mediated interaction between *Aspergillus* spp. and their host seed. They show that seed polyunsaturated fatty acids affect the ability of *Aspergillus* to produce infection structures (spores) and to produce the carcinogenic mycotoxin called aflatoxin. Moreover, *Aspergillus* infection of seed regulates the expression of seed lipid metabolism genes for lipoxygenases (enzymes which produce some of these polyunsaturated fatty acids). The overall goal of this project is to test the hypothesis that linoleic acid (a polyunsaturated fatty acid) and linoleic acid derivatives from seed are virulence factors for successful colonization of seed by *Aspergillus* spp. and possibly other seed-attacking fungi. Confirmation of this hypothesis will lead to strategies to decrease colonization and subsequent aflatoxin contamination of seed by the aspergilli.

Leafy Spurge Genotype Effects on Galling by a Specialized Biocontrol Insect. Biological weed control has been successful in controlling a number of invasive weed species; however, a closer examination suggests that failures are often associated with weed populations that show high levels of genetic diversity.

Dr. Scott Nissen and colleagues of **Colorado State University** and the **University of Nebraska-Lincoln** seek to improve the efficiency of biological weed control by determining the influence of plant genetic diversity and insect co-adaptation on the impact of a natural enemy against an invasive weed. Leafy spurge (*Euphorbia esula* L.) is an invasive species introduced from Eurasia in the 1700's that now infests 2.5 million acres across much of the central Great Plains and Canada. The gall midge, *Spurgia capitigena* Gagné, was one of the many insects released to control leafy spurge; however, the success of the gall midge varies with leafy spurge genotype.

To test the importance of genetic diversity on gall midge success, insects for the original Italian source population and a naturalized population in North Dakota will be given access to a diverse population of leafy spurge plants from across Eurasia and from North America. The investigators predict that the Italian gall midges will be highly successful on Italian spurge but will have varying degrees of success on other genotypes. If naturalized midges have improved success on North American leafy spurge, this will be strong evidence for a shift in population structure of the gall midge to accommodate plant genetic diversity. The results will most likely affect biocontrol management of leafy spurge.

Environmental Stress and Invasion of Native Grassland by Non-native Grasses. Comparisons of invasion of native plant communities by non-native plants suggest that the susceptibility of communities to invasion increases when levels of environmental stress change. Non-native grasses have already largely replaced native grasses across much of the remaining prairies of western North America.

The basic goal of **Dr. Peter Alpert** of the **University of Massachusetts, Amherst** is to test this stress hypothesis, using field experiments in a natural coastal prairie system at the University of California Bodega Marine Reserve and complementary greenhouse experiments at the University of Massachusetts. The coastal prairie at Bodega is especially suitable for the study because it is at a critical, intermediate stage of invasion: non-natives are abundant, but diversity and abundance of natives are still high. Manipulations of nitrogen and water availability and of grazing and burrowing by mammals will test the effects of known stress and disturbance factors in the system on 14 of the most common native and non-native grasses. This project may develop methods to control the spread of invasive plants and maintain biodiversity. For example, land management prescriptions to counter the over-enrichment of native soils by nitrogenous pollutants may turn out to be "chemotherapies" for invaded lands.

Identification and Characterization of Aluminum Tolerance Genes in Grain Crops. Aluminum (Al) phytotoxicity limits crop productivity on acid soils that make up more than 50 percent of the world's arable lands. Genetic-based variation for Al tolerance exists in a number of plant species, yet the underlying molecular basis and the genes for Al tolerance have not been identified.

Dr. Leon Kochian, **USDA Agricultural Research Service**, **Ithaca**, **NY**, aims to elucidate the molecular basis for Al tolerance in important grain crops built upon recent findings concerning a major Al

tolerance mechanism involving Al-activated release of organic acids from the root apex, which in turn binds and detoxifies Al at the root surface. The specific research objectives include the cloning and characterization of Al-activated organic acid transporters in the root apex of Al-tolerant wheat, which mediate organic acid release and thus are candidate Al tolerance genes; identifying wheat Al tolerance genes via comparison of expressed genes in a pair of chromosome 4DL segmental deletion lines that either carry or lack the major Al tolerance gene, and fine scale mapping of Al tolerance in sorghum, with a goal of isolating sorghum Al tolerance genes. The overall objective of this research is to isolate Al tolerance genes to use in the development of crops with increased Al tolerance, to improve crop productivity on the large areas of marginal, acid soils throughout the world.

Identification of Sequences Required for p1 Paramutation. The control of gene expression – and in particular gene silencing – is crucial for plant growth and development and will be key for efficient and controlled genetic engineering of plants. Paramutation is a natural case of gene silencing, which occurs when two particular alleles of the same gene interact and this interaction results in a reduction in gene expression. Paramutation can occur between two alleles of the same gene, between two introduced transgenic loci that share DNA sequences, or between a transgene and a gene that share DNA sequences.

Current data are consistent with shared mechanisms between paramutation and transgene silencing, with both mediated by changes in chromatin structure. In the nucleus, DNA is organized into a complex structure containing numerous proteins, referred to as chromatin. Chromatin structure influences whether or not a gene is expressed. One interesting aspect of paramutation and transgene silencing is that the genes involved can exist in different chromatin structures and expression states, which are transmitted to progeny at high frequencies, but are potentially reversible.

Recently, **Dr. Vicki Chandler**, **University of Arizona**, and colleagues identified a small DNA fragment that is sufficient for paramutation at the *p1* gene in maize. The goal of the project is to perform a more detailed fine-structure genetic and molecular characterization of this region to determine how these DNA sequences mediate paramutation and transgene silencing. A more thorough understanding of paramutation should enable better design of transgenes that will lead to a reduction in the frequency of transgene silencing and more efficient engineering of important crop plants.

Gene Expression in Polyploid Cotton. One of the major processes that have shaped the present composition of plant genomes is gene and chromosome doubling. This often happens by a process whereby all chromosomes become simultaneously doubled. An important consequence of this process is that all genes become duplicated. Theory suggests that after chromosome doubling, pairs of duplicated genes may acquire different and perhaps new functions, or that one member may be eliminated because it is redundant. Despite this well-developed theory, little is known about the dynamics of this process in plants.

The proposed research by **Dr. Jonathan Wendel**, **Iowa State University**, is designed to begin to fill this void using a model system from the cotton genus. Different kinds of chromosomally doubled cotton plants will be studied, permitting comparison of changes that occur immediately after chromosome doubling to those that arise on a longer term time scale. Using new technologies for large-scale screening of gene activity, the research will establish the extent and pace of change of gene activity and functional divergence for hundreds of pairs of genes doubled by the process of chromosome doubling. Some genes may have lost their function, either completely or in specific plant tissues. Other genes may have acquired new function or new tissue-specific activities. Because so many of our most important crop plants have doubled chromosomes and genes, including maize, soybeans, oats, wheat, cotton, and many others, this research is expected to yield important information that has broad applicability to crop improvement.

Cloning of Sh1, a Gene Responsible for Shattering of the Cereal Inflorescence. The long-term goals of this project by **Dr. Andrew Paterson**, **University of Georgia**, are to clone *Sh1*, a gene that eliminates "shattering" of the mature grain crop in many cereals. Cloning of *Sh1* will contribute directly to long-range improvement in, and

sustainability of, U.S. agriculture, by engineering improved genotypes of many existing crops with reduced seed loss at harvest. Many existing and/or prospective crops cannot be fully utilized because of seed losses due to shattering. Cloning of *Sh1* will also help to accelerate commercialization of prospective new crops such as wild rice, canola, spurge, and Vernonia, providing a wider range of options to agricultural producers, and helping to reduce both genetic and environmental vulnerability of the U.S. food production system. Better understanding of the biochemical steps leading to shattering might also suggest new approaches to controlling major weeds such as "Johnson grass" or "red rice." Cloning of *Sh1* may lead to new strategies for weed control, reducing agrichemical use in agroecosystems.

"In my field," Dr. Paterson said, "genomics, NRI grants are among the smaller ones that we compete for, but nonetheless, they are absolutely essential to the national research infrastructure. NRI-funded research 'sowed the seed' for large-scale genomics projects in many major U.S. crops. Looking to the future, NRI-funded research will be essential to 'harvest the fruit' of such genomic endeavors, in the form of basic research outcomes and applied crop improvement."

Ethylene Receptor Signaling. **Dr. Caren Chang**, **University of Maryland**, is looking for the missing links between the perception of the plant hormone ethylene and its immediate downstream molecular actions. Dr. Chang is focusing on the exact relationship between the ethylene receptors and the next component called CTR1, which is the first member of a kinase pathway that sets in motion the myriad effects of ethylene, either formed naturally or applied in agricultural or commercial practice. She is using the model plant system *Arabidopsis*, because of the large number of available mutants and the ease by which mutant forms of the ethylene receptor and CTR1 can be added or subtracted and then the consequences studied. Her project will enable the finding of additional proteins that are involved in this early step of ethylene perception and the examination of how the multiple ethylene receptors interact.

Characterization of a Putative Retinal-based Photoreceptor in Plants. **Dr. Kevin Folta**, a postdoctoral investigator at the **University of Wisconsin-Madison**, has made the outstanding discovery that plants may utilize photoperception very similar to the opsin-based system in animals. Moreover, it opens up a novel blue/green light perception system in plants that could have profound implications in U.S. agriculture. He has found that plants contain a retinal binding protein. Retinal is the chemical moiety of the mammalian photoreceptor molecule, rhodopsin. He will test the critical hypothesis that this protein mediates blue light responses (movements, development, flowering). More than just facilitating an exciting project, the Plant Growth and Development Program is fostering the career of a young investigator destined to make many more major discoveries in plant biology.

Calmodulin, Calcium, and cAMP Gated Ion Channels: Unraveling a Signal Cascade in Plants. **Drs. Gerald Berkowitz**, at the **University of Connecticut**, and **Raymond Zielinski**, at the **University of Illinois**, have teamed up to examine how novel ion channels are involved in a plant's response to invading pathogens – building on a new paradigm in plant disease resistance. Their combined expertise will enable them to express unusual forms of these calcium and potassium channels that are controlled by extracellular nucleotides in frog oocytes to isolate them from other cellular controls. This allows a directed study of their regulation and thus insight into how we might manipulate them to boost plant disease defense in agriculture.

Mechanism and Significance of Ion Mediated Changes in Xylem Hydraulic Resistance. **Dr. N. Michele Holbrook** of **Harvard University** and colleagues are addressing water transport in plants and focusing on the mechanism of water distribution in the xylem. The proposed research is extremely significant as it has the potential to change the concept of xylem function. Xylem vessels are formed from dead cells, yet recent work from this laboratory demonstrates that their hydraulic properties are highly dynamic. The long-held paradigm of xylem function indicates that it is a

passive process with vessels either transporting water with a constant resistance or being blocked due to embolism. Rather than being an assemblage of passive pipes, however, xylem may play a rather active role in regulating water transport.

The investigators have an exciting and innovative idea that ion concentration of the xylem sap affects the porosity of the partitions within the transport pathway (intervessel pit membranes) via the swelling/deswelling of pectins, which are known hydrogels. Furthermore, the xylem ion concentration may be controlled by the phloem, completing the idea of plant control over xylem conductivity. The investigators have proposed a series of field and laboratory experiments to verify the effects of xylem ion concentration on conductivity in several plant species and to test the functional role of the phloem. The results of this research could bring about significant changes in concepts of xylem function. The goal is to understand how ionic effects may contribute to a plant's ability to actively counter losses in xylem capacity due to cavitation as well as to fine-tune their hydraulic architecture in response to rapid changes in their local environment.

Engineering a High-Flux Glycine Betaine Pathway To Enhance Stress Resistance. Drought and salinity are major constraints on U.S. agricultural productivity. Certain plants respond to these stresses by synthesizing and accumulating the protective compound glycine betaine (GlyBet), which helps to reduce stress damage.

Dr. Andrew Hanson, **University of Florida**, is studying engineering of glycine betaine synthesis for enhanced plant stress tolerance. Specifically, this project seeks to genetically engineer the capacity to synthesize GlyBet into crops that lack it. GlyBet is synthesized from phosphoethanolamine (P-EA), and the key enzymes in the pathway are P-EA *N*-methyltransferase (PEAMT), choline monooxygenase (CMO), and betaine aldehyde dehydrogenase (BADH). In earlier work, tobacco that was engineered to over-express these enzymes made some GlyBet, but the amount was limited by the internal supply of P-EA. The investigators now plan to increase the P-EA supply. Plants readily form P-EA from ethanolamine (EA), but it is not clear how they make EA itself. There may be two routes, one via phosphatidylserine (Ptd-Ser) decarboxylase and the other via a unique decarboxylase acting on free serine. Combining serine or Ptd-Ser decarboxylase with PEAMT, CMO, and BADH should allow the investigators to engineer an efficient GlyBet pathway starting from the plentiful precursor serine. The genes that code for serine and Ptd-Ser decarboxylases will be isolated, characterized, and then expressed in tobacco plants that are already overexpressing PEAMT, CMO, and BADH; the impact of the expression of these genes on ethanolamine, choline, and GlyBet synthesis will then be determined.

"Our NRI support over more than a decade," said Dr. Hanson, "has promoted basic understanding of plant stress metabolism, produced patentable discoveries, and propelled seven young scientists into careers in universities, industry, and government. More generally, this support has fostered progress in plant metabolic engineering, a field that in the long term could revolutionize agriculture, the chemical industry, and human health."

Tree Physiological and Growth Responses to Climate Variability Recorded in Tree Rings. **Dr. James Ehleringer**, **University of Utah**, is addressing highly significant questions of climate and elevated CO_2 effects on growth of trees and is using an innovative approach to study these questions. The response of forests to environmental change is significant to both land managers and global change scientists because of their economic value and potential to store carbon. Predicting future forest productivity requires understanding the physiological and growth responses of trees to multiple environmental variables, such as precipitation, temperature, nutrient availability, and atmospheric CO_2 concentration.

Using the record of tree growth and physiology provided by annual growth rings, the investigators will examine the long-term response of forest trees to climate variability and changing atmospheric composition. They will use the oxygen isotope ratio of tree ring cellulose to provide information on the water sources used by trees and the humidity conditions experienced at the leaf surface. In addition, they will use the carbon isotope composition of tree-ring cellulose to provide a record of physiological responses to variation in precipitation

and humidity, and to increasing atmospheric CO_2. Chronologies of tree-ring width and isotope ratios will be employed to (1) extend historical records of summer precipitation, (2) determine physiological and growth responses to precipitation variability, and (3) determine physiological and growth responses to the gradual increase in atmospheric CO_2 concentration.

By extending the records of past summer precipitation, data will be generated that can be used in climate models to clarify the controls on interannual and decadal variation in the intensity of the Arizona monsoon system. From the records of physiological and growth responses to precipitation variability and increasing CO_2 concentration, the investigators can determine the relative importance of these two global change factors in affecting forest productivity over time.

Regulation of Metal Uptake in Plants. **Dr. Erin Connolly**, **University of South Carolina**, is examining metal regulation of protein expression. Certain metals, like iron and zinc, are essential nutrients, and the ability to accumulate these nutrients from the diet is crucial for human health. Current estimates predict that more than 3 billion people suffer from iron deficiency. Plants are the main dietary source of essential metals for much of the world's population. In agriculture, the availability of metals in soil plays a major part in determining crop yields. However, metals may be toxic to plants and animals when present at high levels. Thus, all cells must maintain balanced intracellular concentrations of metal ions and the transport of metals into the cell is a primary control point for the regulation of metal levels.

The goal of this research is to understand how plant cells regulate the uptake of metal ions. The study focuses on the regulation of the Arabidopsis metal transporter, IRT1 (Iron Regulated Transporter). IRT1 was the first identified member of a novel family of metal transporters called ZIPs (ZRT-, IRT-like Protein). Experiments are designed to characterize further the metal-induced post-translational regulation of IRT1. The investigators are using a combined genetic, molecular, and biochemical approach to define the amino acid residues in IRT1 as well as the transacting factors that are necessary for metal-induced regulation of IRT1. These studies ultimately should lead to a better understanding of metal uptake in plants and to the design of plants that are capable of growth on micronutrient-deficient soils and that accumulate metals like iron and zinc and thus have a higher nutritional value.

Flavanoids in Plant Growth and Development. **Dr. Brenda Winkel-Shirley** of **Virginia Tech** will be studying the role of two enzymes, flavanoid 3'-hydroxylase (F3'H) and flavanol synthase (FLS) in flavanoid synthesis and their function. In contrast to the single gene that encodes for F3'H, FLS has multiple tissue-specific isoforms encoded by a multigene family. Dr. Winkel-Shirley will undertake a detailed study of the relative expression patterns of the F3'H and FLS genes and compare the substrate specificities of expressed FLS isozymes. Several enzymes of the flavanol pathway are present as a macromolecular complex and efforts will be made to identify other enzymes, which may interact with the various FLS isoforms. Genetic knockout mutants for FLS will be sought to confirm the substrate specificities and to explore the roles of specific flavanols in UV protection, auxin transport, and plant-pathogen interactions.

Alanine Excretion by Soybean Nodule Bacteroids: Metabolic Fate of Alanine. The line of research in this award to **Drs. David Emerich** and **Thomas Mawhinney**, **University of Missouri-Columbia**, may very well overturn an established dogma. Preliminary results make a compelling argument for alanine (rather than ammonia) being the source of nitrogen exported from bacteroid to legume host and consequently for examining the fate of this amino acid during symbiosis.

Symbiotic nitrogen fixation is the process whereby certain bacteria form an intimate association with specific plants, such as that between *Bradyrhizobium japonicum* and soybean. The association takes the form of root nodules, which are tumor-like growths in the root just under the soil surface. The symbiotic form of these bacteria, called bacteroids, reduces atmospheric dinitrogen to ammonium via the enzyme nitrogenase. The bacteroid-produced ammonium can be used for producing plant proteins in lieu of applied fertilizers. The central issue is how the ammonium moves from the bacteria to the plant. The ammonia diffusion hypothesis has been the generally accepted model of nitrogen transfer

from the bacteroids to the plant host cells. The basis of this hypothesis is that the gaseous form of ammonium diffuses out of the bacteria into the plant cell where it is assimilated first into amino acids, which are the building blocks of proteins.

Experiments are planned to determine whether alanine is the nitrogen-containing compound released from the bacteroids *in planta*. The objectives of this proposal are to determine the symbiotic phenotype of an alanine dehydrogenase mutant, and characterize the alanine transport systems of bacteroids. These objectives are intended to determine the extent to which alanine is able to provide the nitrogen requirement of the plant.

Identity Preservation of GMOs. The consuming public worldwide has a number of perceptions pertaining to genetically modified organisms (GMOs). Some consumers do not differentiate between GMOs and traditional products. Others place a high premium on traditional, non-GMO products. For political reasons, some governments have restrictions on the importation of GMOs, while others do not. Both the perceptions of consumers and the policies of various governments have significant potential impacts on new product development and international trade.

The question being examined by **Drs. GianCarlo Moschini** and **Harvey Lapan** at **Iowa State University** is whether the introduction of a new variety of an agricultural product, which cannot be readily differentiated from pre-existing products, results in different consumer decisions and, consequently, different demands for that product. A product that has an inherent characteristic or marker such that it can be identified through the production, processing, and consumer channels would be considered to have identity preservation. This study examines the potential outcomes, including consumer reactions, if identity preservation were viable. This is the critical issue that Dr. Moschini's team will be addressing, which will have great significance in policy and trade discussions, both domestically and internationally. The usefulness of the study will be to understand consumer reactions and, as a result, the economic implication of alternative policy options.

Outcome 2: A safe and secure food and fiber system

Improving the Health & Well-Being of Poultry. One of the most important well-being issues affecting chickens raised for meat (broilers) are leg disorders. Despite selection by breeders for skeletal strength, approximately 30 percent of broilers have gait disorders severe enough to impair mobility. These disorders make it difficult for birds to access feed and water, are often painful, and cause significant economic loss to the poultry industry, either because the birds have to be culled or because their carcasses are of poor quality.

Dr. Joy Mench and colleagues at the **University of California-Davis** are working to improve this situation. With previous NRI funding, they found that adding simple items to chicken pens that increase bird activity (such as ramps, perches, and dustbaths) improved bird mobility. An NRI renewal award made in FY 2001 will now allow them to continue to evaluate the most promising and economically efficient production method(s) on leg and gait problems, growth and feed efficiency, mortality, fear and stress responses to handling, and meat characteristics. In addition to testing under controlled research settings, pilot testing will also take place at a commercial poultry facility in order to accelerate the extension of these research findings to the poultry industry.

Natural Antibiotics Offer Help for Swine Disease and Food Safety. Widespread use of antibiotics has resulted in the emergence of bacteria that are resistant to antibiotics. Alternatives to conventional antibiotics that will provide effective means of preventing and treating livestock disease, while not interfering with antibiotics used for human health, are urgently needed. One promising answer may be antimicrobial peptides, also known as natural antibiotics. These are small proteins that most animals produce in different parts of their bodies, and which act as a first line of defense against disease at its entry points (e.g., skin, eye, tongue, lungs, gastrointestinal tract).

During the past 6 years, the NRI has supported research studying promising swine natural antibiotics. Specifically, **Dr. Frank Blecha** and colleagues at **Kansas State University**, in

collaboration with researchers at the College of Medicine at UCLA, discovered a natural antibiotic in the tongue of pigs, known as porcine beta-defensin-1. This antibiotic kills bacteria that have developed resistance to conventional antibiotics. The team found that it is even more powerful when used with other pig antimicrobial peptides with which they have been working. An NRI renewal in FY 2001 allows the team to study ways to increase the level of these natural proteins in pigs. By learning how to increase pigs' production of their own natural antibiotics, pigs will be better able to fight diseases using their natural immune systems. The knowledge learned with swine may also be applicable to other farm species (such as cattle and poultry) because these species produce their own natural antibiotics.

Sequencing of Animal Pathogens. In FY 2000, a multi-institutional team led by **Dr. Stanley Maloy** at the **University of Illinois** (and including researchers at the University of Tennessee and University of Minnesota) was funded by the NRI to sequence the genomes of four *Salmonella serovars* (*enteritidis*; *dublin*; *pullorum*; *choleraesuis*). These organisms cause severe disease in cattle, swine, and poultry, and pose food safety hazards to humans. USDA-NRI funding was provided for 3-4x draft coverage of the genomes. By the end of 2001, the sequences of *S. enteritidis*, *S. dublin*, and *S. pullorum* were determined and all sequences were made publicly available (*http://www.salmonella.org*). *S. choleraesuis* will be complete by 2002. The sequence databases have already been widely used by researchers throughout the world studying *Salmonella* animal and human pathogenesis and are being used to guide diagnostic test development. Additionally, the Centers for Disease Control and Prevention is exploring the development of improved identification methods based on the comparative genomic information provided by this project.

Rapid Detection of Microbial Pathogens. A number of projects funded in FY 2001 relate to improving our ability to rapidly detect microbial pathogens or their toxins. **Dr. Robert E. Gawley** at the **University of Miami** received a renewal of a previous grant to develop an optical fiber sensing device for saxitoxin. Consumption of saxitoxin, one of a number of marine toxins that are accumulated by filter-feeding shellfish, can cause illness and death. Currently, a mouse bioassay is the benchmark method for detection of saxitoxin. The goal of this project is to develop a small, portable device requiring minimal sample preparation that will report the presence of saxitoxin by a fluorescence emission transmitted through an optical fiber.

Dr. Avraham Rasooly of the **U.S. Food and Drug Administration** is working on a process to unify identification and characterization of the 14 major microbial foodborne pathogens in a single DNA microarray chip. In addition to the rapid detection of pathogens, this system will allow for analysis of virulence factors and antibiotic resistance genes.

Prevention of E. Coli 0157:H7 Colonization in Cattle. E. coli 0157:H7 is a common cause of bloody diarrhea and life-threatening illness, particularly in young children and the elderly. The Centers for Disease Control and Prevention estimates that this organism is responsible for more than 73,000 cases of illness and 61 deaths per year in the U.S. Outbreaks have been traced to manure-contaminated beef, milk, produce, or water. Prevention of colonization of E. coli 0157:H7 in cattle and its elimination in manure would significantly reduce the number of human illnesses caused by this organism. Two projects funded by the NRI this year are working toward this goal.

Dr. F. Chris Minion at **Iowa State University** is using a technique called Signature Tagged Mutagenesis (STM) to identify the genes responsible for colonization and persistence of E. coli 0157:H7 in the intestinal tract of sheep. These genes can then become targets for future intervention strategies to prevent colonization of this organism in ruminants.

Dr. Rodney A. Moxley at the **University of Nebraska-Lincoln** is studying E. coli 0157:H7 proteins that mediate bacterial attachment to intestinal epithelial cells. He will study E. coli 0157:H7 bacterial strains that differ only by the ability to produce the translocated intimin receptor (Tir) protein to determine if the length of time for fecal shedding in adult cattle varies with respect to strain. He will also determine whether the levels of antibody to Tir are correlated with protection of adult cattle against E. coli 0157:H7, and whether Tir immunization with or without prior recent infection

will boost immunity and further reduce E. coli 0157:H7 fecal shedding.

Poultry: A Food Animal Model for Following Antimicrobial Resistant Enterococci. There is continued concern about the use of antibiotics as growth-promoting agents in food animals and the potential for development of antibiotic resistance in human pathogens. The long-term goal of this study by **Dr. Charles Hofacre**, **University of Georgia**, and colleagues at universities and federal agencies, is to understand the processes involved in the development and spread of resistance in gram-positive bacterial flora of poultry. The investigators will collect microflora samples from commercial poultry farms and processing/slaughter plants for 1 year. The farms will have one house using growth-promoting antibiotics throughout the flocks' life and one house with no antibiotics used. Comparisons of drug resistance genes and plasmids will be made between poultry gram-positive commensals and human enterococci. The human samples will be obtained from the National Antimicrobial Resistance Monitoring System.

Retail Meat Survey for Toxoplasma gondii. *Toxoplasma gondii* is an obligate intracellular protozoan parasite that causes mental retardation, loss of vision, and other congenital health problems in humans and is an increasingly important cause of mortality and morbidity in the immuno-suppressed. The Centers for Disease Control and Prevention lists *T. gondii* as one of three pathogens, which account for over 75 percent of all deaths due to foodborne disease in the U.S. Although cats have long been thought to be the main source of human exposure, the Centers for Disease Control and Prevention now estimates that up to 50 percent of all human infections result from the ingestion of infected meat. However, little is known about the prevalence of this parasite in retail meats. The goal of proposed research by **Dr. Jitender Dubey**, **Agricultural Research Service**, **Beltsville**, **MD**, and colleagues is to determine the prevalence of *T. gondii* in three major commodity meats (beef, chicken, and pork). Samples will be collected over an 18-month period from a sampling method representing 80 percent of the U.S. population. Strains of *T. gondii* will be isolated from meat, genetically typed, and compared with isolates from humans.

Clonal Dissemination of Antimicrobial Resistant Campylobacter jejuni and Escherichia coli in Cattle. There is an increasing concern that antibiotic resistance in both pathogenic bacteria and the normal flora presents a risk to the public health. Reduction in the degree of antibiotic resistance is an important public health goal. The antibiotic-resistant flora that appear after antibiotic exposure of cattle and other food animals may be 'new' antibiotic-resistant strains originating on the farm, or may be pre-adapted strains that originated elsewhere and were transferred to the farm by animals, feed, water, wildlife, humans, or other mechanisms. The origin is important, since different origins require different control measures. For *Salmonella typhimurium*, wide dissemination of antibiotic-resistant strains is the predominant process.

Dr. Thomas Besser, **Washington State University**, and colleagues will look at whether wide dissemination of antibiotic-resistant strains is also important in *Campylobacter jejuni* and *E. coli* in the bovine intestine. In addition, this study will determine whether antibiotic-resistant *E. coli* can be competitively displaced by non-antibiotic-resistant strains. These studies will provide basic information about the role of clonal dissemination in the epidemiology of antibiotic resistance on cattle farms, and so aid risk assessment of commensal flora with antibiotic resistance and prediction of effective interventions to reduce antibiotic resistance frequency.

Outcome 3: A healthy, well-nourished population

Building Stronger Bones with More Digestible Calcium Supplement From Milk. The U. S. population continues to be at high risk for calcium deficiency, and consumer recognition of this risk is high. Therefore, a need exists for calcium-fortified food products that will provide larger amounts of calcium in a single serving. Caseinophosphopeptides (CPPs) derived from bovine milk have the ability to bind minerals, thereby enhancing calcium utilization. CPPs derived from polymorphic caprine (goat) casein appear to cause enhanced calcium solubility. Solubility may influence the amount of calcium available for absorption by the human body. Researchers at **Prairie View A&M University**, led by **Dr. Adela Mora-Gutierrel**, are

studying the uptake and retention of polymorphic caprine casein to determine the nutritional benefits. Results could lead to the production of innovative protein ingredients and applications in the dairy and nondairy food industries as calcium fortification systems.

New Technology for Purifying and Improving Soy Products. Soy has long been recognized as a healthy and inexpensive source of protein. A research team at **Iowa State University**, led by **Dr. Patricia Murphy**, is experimenting with new ways to process soy protein ingredients to enhance health benefits. Their goal is to optimize and improve purification systems for the soybean proteins, glycinin and conglycinin, while maintaining healthy phytochemicals. They are also studying the potentially valuable non-protein products, the isoflavones and saponins for alternative food uses. Successful completion of their goals could lead to valuable soy protein products with additional health benefits for U.S. consumers and expanded markets for U.S. producers.

Improving the Nutritional Status of Older Women. Interest in maintaining the health and nutritional well-being of older adults (65 and over) is gaining importance as this population continues to expand from 12 to 22 percent of the total population in the U.S. by 2030. Several recently awarded NRI projects are focusing on improving the nutritional status of older women.

Two groups of researchers at the **University of Connecticut**, headed by **Drs. Jasminka Ilich-Ernst** and **Karen M. Prestwood**, are studying the effects of dietary intake on bone health in women over the age of 60 years. Dr. Ilich-Ernst is testing whether older women (ages 60 to 85 years) with higher dietary sodium intakes (e.g., their usual intake of about 3.5 g sodium/day) will lose more bone mass over a 2-year period in different skeletal sites, compared to women with lower dietary sodium intakes (about 1.2 g/day). Dr. Prestwood will look at the effects of dietary soy protein, with or without supplemental isoflavones, on the bone mass of women over the age of 70 years. Results of both studies will be used in formulating dietary recommendations for older women.

Dr. Naman Ahluwalia at **Penn State University** is determining the effects of iron deficiency on immune function in homebound older women (age 60 years and older). She is also evaluating the functional benefits associated with supplementation to improve iron status in this population. In a previous NRI grant, she found a high prevalence of iron deficiency (19 percent) and anemia (15 percent) among homebound older women. Results of the current study will be used to make recommendations for testing for iron deficiency and follow-up to improve iron status in this vulnerable group.

Improvement in Dietary Standards. Standards for dietary nutrient intakes, such as the Reference Dietary Intakes issued by the Institute of Medicine, are widely used by nutrition and health professionals to assess dietary intakes of populations and make recommendations. There is a continuing need to add to the research base of information used to formulate such recommendations. Often, research is lacking in vulnerable groups such as infants, children, adolescents, and pregnant and lactating women. Recommendations for these groups must be extrapolated from research conducted in adults. In other cases, the form in which a nutrient is consumed affects its bioavailability; data may be available for one form, but not for others. Two studies funded by NRI in FY 2001 will contribute to the research base available for formulating dietary standards in the future.

Dr. Joseph R. Prohaska at the **University of Minnesota-Duluth** is working on determining the copper requirement necessary to support optimal human development. He has shown, in previous studies in copper-deficient rats, that the activity of the copper-dependent enzyme peptidylglycine alpha-amidating monooxygenase (PAM) may be a useful indicator. In this project, he will investigate the utility of PAM as a marker for humans using a combination of experiments in copper-deficient rats and studies using human serum from subjects of various age groups and copper status states. These studies will aid in the establishment of a biochemical marker of nutritional copper status.

Dr. Marie A. Caudill at **California State Polytechnic University** is evaluating the effectiveness of natural dietary folate in improving folate status in

non-pregnant women. Current recommendations from the Institute of Medicine are that all women of childbearing age consume 400 g/day synthetic folic acid to reduce the risk of neural tube defects in the fetus. Those recommendations focused on synthetic folic acid because limited data were available on the equivalency of absorption of dietary folate as compared to the synthetic form, and because studies of the effectiveness of dietary folate in improving folate status were inconclusive. The results of this study can be used in formulating future recommendations for folate intake for women of childbearing age.

Outcome 4: Greater harmony between agriculture and the environment

Enhancing Safety of Biocontrol with Bacillus cereus. Biocontrol of agricultural pests and pathogens is an attractive alternative to synthetic pesticides. However, as more microbial biocontrol agents have reached the final stages of regulatory review, the relatedness of certain biocontrol bacteria and human pathogens has begun to generate concern among regulators and the public. As biocontrol becomes more pervasive, so do any attendant risks. The problem is well illustrated by the spore-forming bacilli, many of which are highly effective biocontrol agents. *Bacillus thuringiensis* strains have been successfully marketed for 30 years for insect control, a *B. cereus* strain was recently registered as a biopesticide on cotton, and other *B. cereus* strains are under review as biocontrol agents for crop disease. Most strains of *B. thuringiensis* and *B. cereus* produce a mammalian enterotoxin associated with food poisoning.

In NRI-funded research, **Dr. Jo Handelsman** at the **University of Wisconsin-Madison** will remove the genes for enterotoxin production and test the behavior of the mutant in the field. She has successfully removed two genes encoding enterotoxins in a previous NRI-funded project. A third gene will be removed by constructing a deletion mutant. The mutant will be applied to soybean seeds at planting and tested for the ability to grow on soybean plants and enhance yield. This research will provide a rational basis for regulatory decisions about registration of *B. cereus* strains as crop inoculants.

Improved Utilization of Nutrients Reduces Excretion in Animal Wastes. The efficiency of nutrient utilization in the gastrointestinal tract is a critical factor determining the economic and environmental cost of livestock production. If not utilized for synthesis of body tissues or for production, essential nutrients such as nitrogen and phosphorus are voided through excretion in animal wastes, thereby contributing significantly to deposition of these nutrients into the environment. **Dr. Alexander Hristov**, **University of Idaho**, has demonstrated that modifying the carbohydrate composition of the diet can enhance ruminal ammonia nitrogen utilization in cattle. He expects to enhance, through dietary means, the capture of dietary nitrogen into milk protein in dairy cows and also effectively reduce nitrogen excretion. This may offer practical tools to manipulate the efficiency of dietary nitrogen utilization in dairy cows and could lead eventually to more efficient utilization of feed protein and reduced release of nitrogen into the environment.

Within the aquaculture industry, phosphorus that is not retained by the fish is lost in the fecal matter and urine, and effluents from aquaculture facilities frequently contain excessive phosphorus from fish waste that leads to unwanted growth of algae in nearby lakes and rivers. **Dr. Nichole McDaniel**, a Postdoctoral Fellow at the **University of Medicine and Dentistry of New Jersey**, is studying how trout absorb and retain phosphorus from the diet. The long-term goal of this research is to enhance utilization of dietary phosphorus in trout by elucidating the mechanisms regulating absorption of phosphorus by the intestine and resorption by the kidney. In doing so, these investigators hope to be able to improve phosphorus uptake and retention, thereby making it possible to add less phosphorus to trout diets while supporting maximum fish growth. This may contribute eventually to the reduction in phosphorus level in the environment.

Identifying the Sites Vulnerable to Agrochemical Contamination. Research is being supported that will better predict or screen sites that are sensitive to contaminant transport using new and novel approaches. **Drs. John M. Norman**, **Francisco J. Arriaga**, and **Birl Lowery** of the **University of Wisconsin-Madison** are developing

an innovative geostatistical 3-dimensional landscape-based approach for quickly creating a soil data set within a watershed or closed basin for assessing and modeling water and solute fluxes.

Dr. Chittaranjan Ray at the **University of Hawaii at Manoa** will use an approach that allows for simultaneous determination of chemical transport and hydraulic properties using a simple method with drip irrigation emitters at multiple site locations. He will employ artificial neural networks for vulnerability assessment using occurrence data of nitrate and pesticides, well site land use information, well construction details, and other climatic, hydrologic and geologic data. Once more pesticide and nitrate occurrence data become available along with site information, the neural network model can be used to predict contamination potential for new sites with adequate site and hydrologic data.

At the same time, **Drs. H. Don Scott**, **B. Dixon**, **A. Mauromoustakos**, **J.V. Brahana**, and **J.C. Dixon** of the **University of Arkansas** will use Neural Networks, Fuzzy Logic, and a newly developed Neural-Fuzzy model, all on a Geographic Information System platform, to predict areas where the groundwater is vulnerable to contamination by agricultural chemicals. These new techniques were chosen because they provide robust but economically feasible tools to generate groundwater vulnerability maps for policy makers. This research will use existing state and federal digital data for soils, land use, geology, and water quality data from 450 geo-referenced wells in the Mississippi Delta region of Arkansas. All three projects will lead to the identification of high-risk sites that can be sampled for confirmatory contamination by planners and by health or regulating agencies. The well users can then be informed about their water quality. Considerable cost savings can be realized by testing only the most vulnerable sites.

Pesticide Degradation. Because of their widespread use, acetanilide herbicides, such as alachlor, are a ubiquitous source of contamination of soil and water resources. Contamination of groundwater by these herbicides is of particular concern because these compounds are potentially carcinogenic and exposure to them may cause serious health problems.

Drs. Jay Gan and **S.R. Yates** at the **University of California-Riverside** discovered a novel reaction in which chloroacetanilide herbicides were dechlorinated and detoxified by thiosulfate salts. Addition of ammonium- or sodium thiosulfate to herbicide-contaminated sand columns reduced herbicide leaching by up to 99 percent. Because common thiosulfate salts are fertilizers or otherwise inexpensive products, this finding has great promise for practical implementation in many contaminant management scenarios, such as for wastewater treatment, spill cleanup, container decontamination, and remediation of polluted aquifers. A new project will evaluate the feasibility and conditions of using thiosulfate salts to decontaminate acetanilide herbicide residues from soil, water, and aquifers. Development of this application will provide an effective tool for preventing and remediating environmental contamination of a number of heavily used herbicides.

Pathogen Tracking and Reduction in the Environment. Several projects are aimed at tracking, identifying, and evaluating transport and environmental attenuation of human pathogens. Groundwater contaminated with pathogenic microorganisms has been implicated in more than 70 percent of all waterborne disease outbreaks in the U.S.

Drs. Yan Jin, **Donald L. Sparks**, and **Abraham M. Lenhoff** of the **University of Delaware** will examine potential colloid-mediated transport of viruses. Sources of groundwater contamination with viruses include septic tanks, private and municipal waste-disposal systems, and land application of sewage sludges and animal wastes. Viruses can travel long distances in soil and groundwater aquifers and pose a public health threat at very low concentrations. Thus, it is critical to understand all the processes and mechanisms controlling their survival and transport in the subsurface to establish regulations that are protective of public health.

Using avian pathogens as model viruses, this project will integrate experiments at both microscopic (molecular) and macroscopic (batch and column) scales, to systematically investigate the three major processes that potentially contribute to the importance of colloid-facilitated virus transport: (1) virus association with colloids, (2) virus survival charac-

teristics in the presence of colloids, and (3) co-transport of viruses and colloids under various environmentally relevant conditions. Information obtained will ultimately lead to prediction of virus transport under a variety of soil and environmental conditions.

Cryptosporidium parvum is a human pathogen that is of particular concern. Recent evidence suggests that pathogen transport in streams is mediated by interactions with suspended and bed sediments. **Drs. Thomas Harter**, **A.L. Packman**, **E.R. Atwill**, and **C.F. Brush** at the **University of California-Davis** will examine the in-stream attenuation and net downstream transport of viable *C. parvum* oocysts in surface waters. Innovative laboratory experiments will specifically examine *C. parvum* association with natural sediments, deposition in streambed sediments, downstream transport, and the effect of suspended particle interactions on viability in streams. On the basis of these experiments, tools will be developed to predict the net reduction of viable *C. parvum* oocysts between upstream agricultural discharge points and downstream surface water supplies.

Another project in this area is by **Drs. Roderick I. Mackie**, **R.I. Aminov**, **I.J. Krapac**, and **J.C. Chee-Sanford** at the **University of Illinois**, who address the problem of bacterial contamination of groundwater by swine production lagoons. These storage structures can leak or seep contents into the ground beneath, creating a risk for bacterial contamination of the underlying groundwater system. They will study groundwater fecal contamination, including pathogenic bacteria, and determine if the contamination may be traced to agricultural origins. This project will use molecular based methods to monitor the presence and distribution of fecal indicators in groundwater underlying two swine production facilities with different geology. The researchers propose a new molecular fingerprinting method using antibiotic resistance gene profiles to trace bacterial contaminants in groundwater to an animal production facility. These molecular-based methods will be more rapid, result in higher sensitivities for detection of specific viable bacterial species, and provide genotypic information that culture-based methods lack.

Another project by **Drs. Valerie J. Harwood**, **Charles Hagedorn**, and **Bruce A. Wiggins** from the **University of South Florida** will use Bacterial Source Tracking (BST) to identify the source of fecal bacteria contamination. BST methods require (1) a reproducible "fingerprinting" technique to differentiate strains of indicator bacteria such as *Escherichia coli* and (2) generation of a representative library of indicator bacteria fingerprints from the animal and human sources that may impact waters. Knowing the sources of microbial pollution in water is important to accurately assess risk, to identify and eliminate point source pollution, and to establish land management practices that decrease contamination from nonpoint sources. The project compares three BST methods using *E. coli* and *Enterococcus*; one based on antibiotic resistance analysis, and two based on DNA fingerprints (ribotyping and pulsed field gel electrophoresis) as to accuracy, utility, practicality, and cost.

Carbon, Nitrogen, and Global Change. Carbon and nitrogen storage and release from soils and waters are important processes in mediating the concentrations of greenhouse gases in the atmosphere. This is an important topic addressed by several projects. **Dr. H. Curtis Monger** at **New Mexico State University** has demonstrated the importance of inorganic carbon sequestration and release in arid soils. **Drs. Dianne Ahmann** and **Junko M. Marr of the Colorado School of Mines** studied effects of elevated CO_2 on the fluxes of methane, a potent greenhouse gas, to and from the soil and found that elevated atmospheric CO_2 caused a 25 percent decrease in methane uptake by the soil, while increasing soil moisture in forest soils.

Knowing how much additional carbon is stored in soil because of carbon dioxide fertilization will lead to more accurate predictions of future CO_2 levels. **Dr. Kevin G. Harrison** at **Boston College** seeks to determine if CO_2 enrichment increases soil carbon storage, using a method he developed for determining the soil carbon CO_2 fertilization factor (the fractional change in soil carbon input divided by the fractional change in carbon dioxide level) using radiocarbon, ^{13}C, and carbon inventory measurements. It is hoped that the techniques used for this research will be applied to other CO_2 fertilization

experiments and used to evaluate how much carbon might be sequestered in soil by re-vegetating abandoned agricultural land. If a type of vegetation had an unusually high soil carbon CO_2 fertilization factor, plantations of this species could be established on abandoned cultivated land to further slow the build-up of atmospheric carbon dioxide levels.

Drs. George F. Vance, **Peter D. Stahl**, **Jeffrey M. Welker**, **J.T. Farenstock**, **J.A. Morgan**, and **G.E. Schuman** of the **University of Wyoming** seek to advance the fundamental understanding of soil biological processes, especially the synchrony between soil nitrogen (N) dynamics and CO_2 flux, while providing insight as to how land management may alter soil carbon (C) sequestration. They will address the temporal coupling of soil N processes (N mineralization, immobilization) with net CO_2 exchange and long-term soil C storage, and study the effects of grazing on microbial biomass production, N mineralization, soil organic matter traits (labile vs. recalcitrant soil C) and annual budgets of net CO_2 exchange in mixed-grass prairie, an alpine grassland, forest meadow, and sagebrush shrubland grasslands, which when combined represent more than 50 percent of western U.S. grazed lands.

Tools for Better Models and Basic Understanding of Soil and Water Properties. Management of crops, rangelands, and forests, as well as many models for crops, chemical transport, hydrology, gas exchange, and nutrient availability, rely on an understanding of soil structure, surface area, water status, and water flow. Soil water measurements by electromagnetic methods including time domain reflectometry (TDR) are extensively utilized in agricultural research and management.

Drs. John M. Wraith and **Dani Or** of **Montana State University** developed a new physical model of soil dielectric behavior that considers bulk water and water bound in thin water films to soil surfaces and the response of these two types of water to changes in temperature. Research will further develop this model to better define the parameters and give a more explicit treatment of dielectric properties of bound water. This thermodielectric phenomenon may be exploited to obtain measurements of wettable specific surface area of soils and other porous media. Specific surface area is critical to behavior of soil water and agricultural chemicals, and to myriad soil microbial processes, yet is underutilized due to lack of simple and accurate measurement methods. It is hoped that this study will lead to simple correction factors for water content measurements using TDR, capacitance, and related methods, and a new method for noninvasive determination of specific surface area of soils.

Soil hydraulic properties are the key information needed to quantitatively describe water flow and chemical transport in soils and groundwater systems. However, hydraulic properties of natural soils are scale-dependent and spatially variable. The scale-dependency, variability, and heterogeneity of soil properties and their impact on transport processes in soils are poorly understood. The main objective of a new project by **Dr. Renduo Zhang** of the **University of Wyoming** is to measure soil hydraulic properties at different locations and different scales in large fields to develop scale-dependent relationships of soil hydraulic properties. He will also characterize spatial variability and heterogeneity of soil hydraulic properties as a function of measurement scales and their impacts on transport processes in heterogeneous soils. These data, combined with information from a FY 2000 project by **Drs. Dani Or** and **Markus Tuller** at **Utah State University**, who have a pore scale model to be verified by using high-resolution microscopy, will provide essential information for predicting water movement, solute transport, and general water-plant relations.

Nitrogen Dynamics. Nitrogen dynamics in soils and water is a common topic due to the importance of nitrogen in its various forms as a plant nutrient, water contaminant, potential greenhouse gas, and a primary cause of eutrophication, contributing to such problems as the dead or hypoxic zone in the Gulf of Mexico.

Dr. David B. Dail of **Penn State University** showed that in the upper layers of forest soils in Pennsylvania there can be rapid transformation of added nitrate and nitrite to organic forms. The study concluded that 40 percent of the nitrate was immobilized via an abiotic mechanism. For nitrite, 40 percent was immobilized to an organic form, while 30 percent was lost to the gaseous form via abiotic denitrification.

Drs. Jennifer L. Tank, **Mark B. David**, and **Todd V. Royer** at the **University of Notre Dame** and the **University of Illinois** will be measuring rates of denitrification (i.e., nitrogen loss) in several small agricultural streams in the Midwest, with the goal of determining the relative importance of denitrification in removing nitrogen from these water bodies. Additionally, they will examine the environmental factors that might limit the potential of denitrification to remove nitrogen from small streams. It is thought that other processes occurring in these streams, such as photosynthesis by algae, may be restricting the amount of nitrogen being removed via denitrification. If so, current estimates of nitrogen loss from agricultural watersheds will likely be in error.

Drs. Francis L. de los Reyes III and **Jiayang Cheng** at **North Carolina State University** will study the fundamental microbiology and performance of a novel biological treatment system that has shown promise as an alternative to manage excess nitrogen in swine wastewater treatment lagoons. A key component of the treatment technology is a reactor that alternates between aerated and non-aerated conditions. This operation allows microorganisms to convert ammonia to nitrate, and then subsequently convert nitrate to harmless nitrogen gas within a single reactor. The research approach will combine molecular (nucleic acids-based) methods for identifying and quantifying key microbial groups with controlled lab-scale reactor studies. This combined molecular and engineering approach will increase our fundamental understanding of the process, which will in turn lead to better design and operation guidelines and more effective adoption of the technology.

Transition to Conservation Tillage. Conservation tillage has the potential to decrease agricultural production costs; improve yields by decreasing compaction; increase labor and energy efficiency; increase carbon sequestration and decrease emissions; improve air, soil, and water quality; and provide more and higher quality habitat for wildlife.

Dr. Steven Temple and colleagues at the **University of California-Davis** will study the transition to conservation tillage in irrigated organic and conventional farming systems. To measure changes in the systems over time, they will begin by collecting baseline data on a variety of biological and physical factors, using these data to understand how conservation tillage affects the different farming systems, and how the systems affect their external environment. These analyses will provide a foundation for future studies of the total costs and benefits of different farming systems to farmers and society.

Salmon Habitat Enhancement. One of the primary environmental issues associated with agriculture in the Pacific Northwest is the degradation of salmonid habitat. **Dr. Jun Jie Wu** and colleagues at **Oregon State University** have received funding to integrate biological, physical, and economic models to determine the optimal targeting of conservation efforts for salmonid habitat enhancement in the Grande Ronde basin of Oregon. The integrated modeling system will consist of a physical model that links physical and riparian characteristics to water quality indicators, a biological model that links water quality and riparian conditions to salmonid abundance, and an economic model that estimates economic efficiency of alternative targeting criteria to enhance salmonid production. Results of the research will be critical for improving the design and implementation of conservation programs in this region.

Outcome 5: Enhanced opportunities for farmers, ranchers, and rural people and communities

New Eco-Friendly Bio-Plastics and Bio-Composites. New environmental regulations, societal concerns, and growing environmental awareness have triggered the search for new products and processes that are compatible with the environment. Bio-composite materials made from natural/bio-fibers and bio-plastics can supplement and eventually replace petroleum-based composite materials for structural applications offering new agricultural, environmental, manufacturing, and consumer benefits.

Dr. Lawrence Drzal and colleagues at **Michigan State University** are developing a new technique called Bio-Composite Stampable Sheet (BCSS) Processing that will convert engineered natural fiber (ENF) and soy-protein-based bio-plastic into sustainable bio-composites. This new process will

achieve a bio-composite that is at least equal in performance and lower in cost to glass-reinforced composite materials for structural applications. The success of this project would supplement the use of petrochemical-based materials with renewable resource materials and improve the long-range benefits to agriculture, the environment, industry, and society.

Stuck on Soy: A Source of Wood Adhesive. Wood composites currently rely heavily on petroleum-based adhesives such as phenol-formaldehyde and urea-formaldehyde resins. However, petroleum resources are naturally limited, even though there is no shortage of petroleum in the near future. In addition, formaldehyde that is emitted in the production and the use of wood composites is hazardous to human health. The development of a formaldehyde-free wood adhesive from a renewable resource is expected to have great impacts on the continued growth and success of the forest products industry and on the living environment. A marine adhesive protein secreted by marine organisms such as mussels is one of the strongest and the most water-resistant natural adhesives, but it is not readily available economically. **Dr. Kaichang Li** at **Oregon State University** is investigating the conversion of soy protein to a strong water-resistant wood adhesive using a marine adhesive protein as a model. Soy proteins are readily available, inexpensive, and annually renewable, so the completion of the project could yield an inexpensive, healthy, and safe natural alternative.

Corporate Farming: Do State Laws Work? Are state laws that restrict corporate farming effective? Are they in the best long-term interests of competitiveness in the agricultural sector? Or, will they eventually result in a less competitive agricultural sector?

A study being led by **Dr. Azzedine Azzam** at the **University of Nebraska** will examine laws passed in Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, Oklahoma, South Dakota, and Wisconsin that restrict "corporate farming." Basically, such laws make it illegal for corporations, other than family farm or family ranch corporations, to own real estate employed in agricultural production. Proponents of these laws believe they are appropriate means by which family-owned and family-operated farms and ranches are promoted. Others, however, believe that such laws tear at the competitive fabric of agriculture that eventually will result in such "family farms" becoming economically nonviable. The question being examined by the research team is to assess the relative importance of each of these questions relative to the cattle industry. Do the "anti-corporate" laws have an adverse impact on the evolution of feedlot size and structure in the major cattle feeding states? The study will provide farmers, ranchers, voters, and policy makers with a scientific assessment of policy measures for which the effect on the competitiveness of agriculture is yet to be fully understood.

Welfare Reform: The Impacts on Rural America. In 1996, the Congress enacted and the President signed one of the most far-reaching welfare reform acts ever. The Personal Responsibility and Work Opportunity Reconciliation Act (PRWORA) was designed primarily to move people from welfare roles and into employment. A key component of the legislation limited welfare recipients to 5 years of welfare. There are many ways to assess the impacts of this legislation, and a number of complementary projects were funded to do so. One aspect of the Act was to increase the responsibilities of state and local governments while reducing those of the federal government.

Dr. Linda Lobao at the **Ohio State University** and her colleagues are examining the impacts in terms of the increased fiscal responsibilities and burdens placed on county governments throughout the nation as a result of PRWORA. The study is being conducted in concert with the National Association of Counties.

Dr. Jill Findeis and colleagues at **Penn State University** are examining the experiences with working or the process of leaving poverty behind. Their focus is to understand the differences regarding the impacts of the 1996 Act on employment in rural versus urban areas. The project is designed to be complementary to the Mellon Community Bridge Project, a cooperative extension training and support pilot program in Pennsylvania designed to help recipients make the welfare-to-work transition. Determining these differences will help in designing more effective transition programs.

Dr. Kathleen Ann Pickering at **Colorado State University** and colleagues are examining welfare reform impacts on rural poverty in four areas of the country where persistent rural poverty has existed for years. The demographic makeup of these four areas – Appalachian Kentucky, the Mississippi Delta, the Lower Rio Grande Valley of Texas, and Indian reservations in South Dakota – will permit the examination of impacts and the role of race, ethnicity, class, and gender in defining their respective experiences resulting from welfare reform.

A fourth study, led by **Dr. Domenico Parisi** at **Mississippi State University** and colleagues, is examining welfare reform in the context of participation in the Temporary Assistance for Needy Families (TANF) Program. In 2002 (the sixth year of the Act), federal guidelines require the greatest reductions in the TANF roles. The research team is basically examining the extent to which characteristics of clients and communities across urban and rural regions affect TANF dynamics, and the alternative activities by which recipients leave TANF.

A final study within this welfare reform analysis portfolio is a multi-regional effort led by **Dr. Jean Bauer** at the **University of Minnesota**. Dr. Bauer and her research team are examining the well-being of rural low-income families in the context of welfare reform brought about by the 1996 Act. These studies collectively should provide an excellent assessment of the impacts of welfare reform on both urban and rural residents and differences between the two, with the expectation that the findings will lead to appropriate adjustments to the 1996 Act.

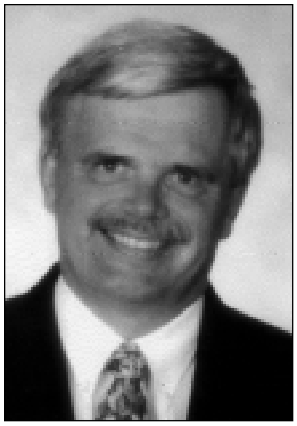
President's Early Career Award for Scientists and Engineers (PECASE)

Dr. Daniel Strawn of the University of Idaho was the FY 2001 recipient of a 2001 Presidential Early Career Award for Scientists and Engineers (PECASE) for CSREES-NRI. Dr. Strawn was recognized by the NRI for the current and potential future excellence of his research. Dr. Strawn's grant is entitled "Molecular Structure of Inner-Sphere and Aqueous Multinuclear Pb(II) and Cu(II) Complexes on Clay Minerals." This work on lead and copper complexes was recognized by the review panel as being cutting-edge basic soil science in that it will improve our understanding of the interactions that control availability of these two common contaminants to microorganisms and plants, as well as their mobility in the environment. This grant addresses national priorities related to remediation of contaminated soils and management of metals in the environment. As a result of the PECASE award, Dr. Strawn's grant is extended to 5 years, enabling him to devote more time to his research during this critical phase of his career.

Dr. Strawn's research will look at the retention of lead and copper in soils at the molecular level, beginning with pure mineral soil components using electron spin resonance spectroscopy and X-ray absorption fine structure spectroscopy. This will provide experimental evidence on the factors controlling the types of adsorption behavior observed, and the mechanisms involved. Results will be used to gain a better understanding of molecular retention processes, which are expected to lead to a new conceptual model that will allow for improved predictions of the form and bioavailability of toxic metals in soils. The extension of time and money from this award will allow Dr. Strawn to extend these experiments to whole soils or whole soil clay fractions, and to investigate the kinetics of the different adsorption mechanisms, making the results more immediately applicable to real-world problems and their management.

From Discovery to Commercialization: A Success Story for the Competitive Grants Program (CRGO/NRI) of the United States Department of Agriculture

Diagnosis and Therapy for an Important Type of Subfertility



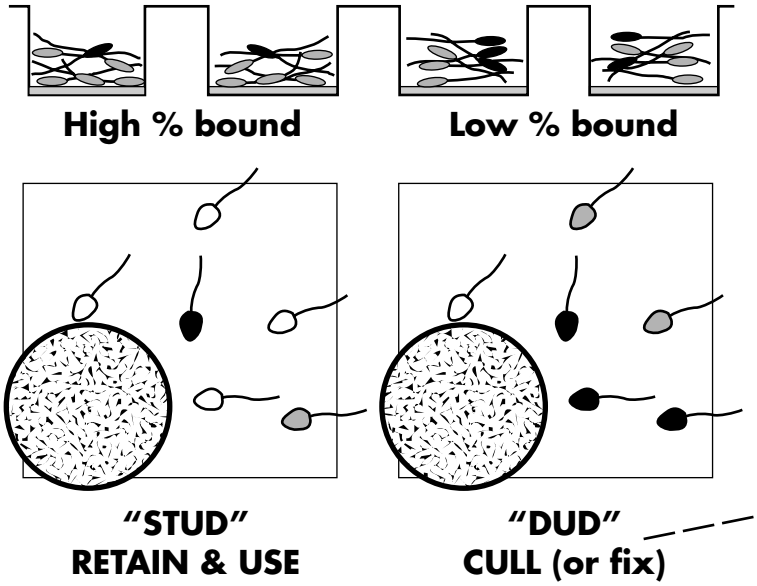
Professors Roy H. Hammerstedt and **Guy F. Barbato**, of Penn State University, received multi-year NRI support in the early 1990s to study components of rooster sperm essential to sperm-egg binding, because frozen-thawed sperm rarely fertilized eggs. In chickens, the target of fertilizing sperm is the membrane surrounding the yolk of an egg. They found that an extract of this membrane could be used as the substrate for a laboratory assay for sperm binding, using a microwell plate. This assay allowed study of synthetic peptides to find one mimicking the native molecule involved in initial sperm-egg binding.

Disclosures to Penn State resulted in four U.S. patents for a sperm-binding assay and a pro-fertility peptide. The technologies were licensed to BioPore Inc., a small business in State College, PA, founded in 1987 by Hammerstedt, Rupert P. Amann, and Sandra R. Hay. Based on data from the NRI projects, BioPore applied for and received USDA and NIH SBIR awards to develop the sperm-binding assay for poultry and humans, and with internal funding it also showed that the assay had utility with boar, bull, and stallion sperm. Males whose sperm were inferior in the sperm-binding assay, i.e., DUDs, were likely to be subfertile. Importantly, fertility of most DUDs and many "normal" males can be improved by exposure of their sperm to a synthetic peptide before use for artificial insemination.

The nature of the two-pronged technology resulting from the original NRI research is illustrated on the next page. In marketing the DudFinder® Sperm-Binding Assay, BioPore is targeting the pig industry because of the high return from eliminating subfertile boars, with turkeys as a second market. Application studies with FertPlus® Peptide have confirmed utility with cattle, pigs, roosters, and turkeys, but commercial introduction has been delayed while production is scaled up. FDA approval for human applications is being sought, with final evaluation of the comparable ParentPlus® Sperm-Binding Assay to have been completed in early 2002 and a trial with FertPlus® Peptide in preparation. Use of these two technologies, directly evolved from NRI research, will benefit cattle, pig, and turkey producers in 2002.

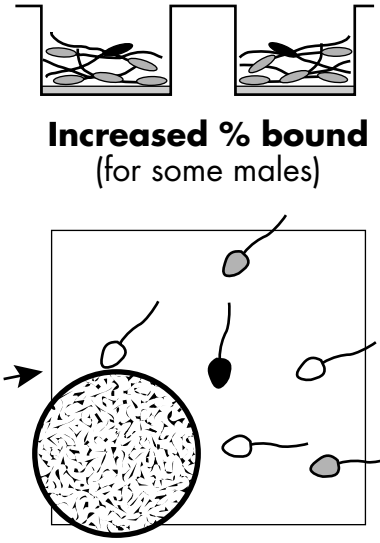
DudFinder® Sperm-Binding Assay Plate

Evaluate group of males; classify each male



FertPlus® Peptide

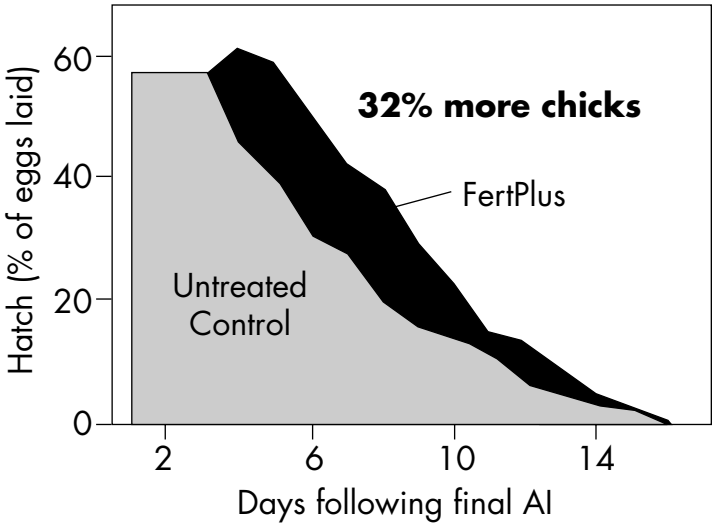
Therapy for faulty binding



Benefit from exposure of sperm to FertPlus® Peptide

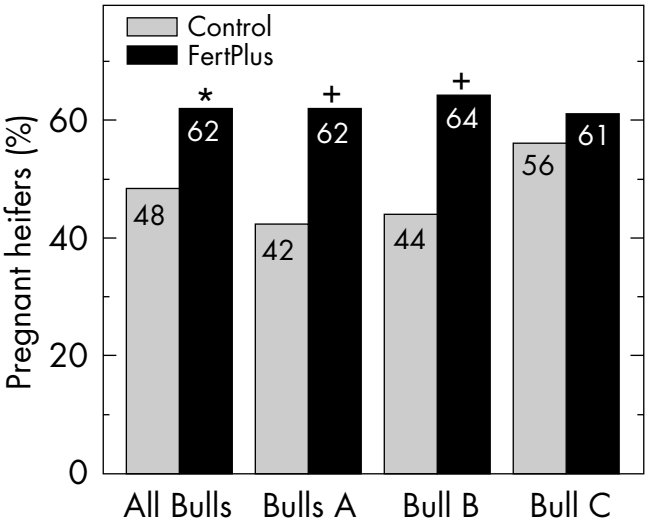
Chicken

Effective for 10 days after AI

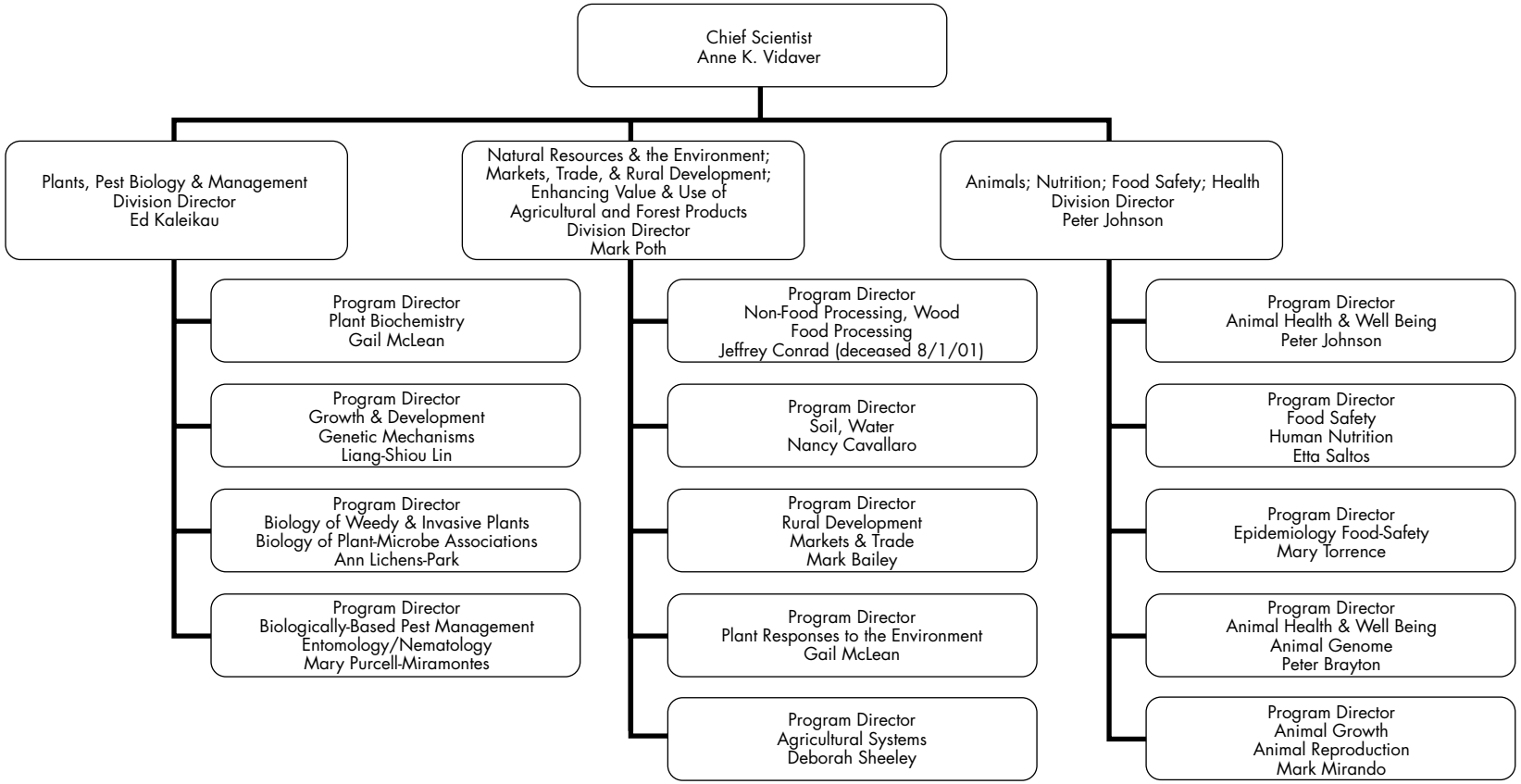


Bull

Effective after AI



National Research Initiative Competitive Grants Program



Assistant Program Director
Jason Hitchcock

Program Specialist
Dorothy Wilkins

Program Assistants
Pamelia Carter
Roderika Davidson
Betty Wilder

Grants Support Assistants:
Georgette Clark
Karen Ebron
Sharrell Edwards
Lisa Ensley
Theresa Ford
Jennifer Hyde
Lori Meaney
Alexandra Raver
Shawnette Rice-Cranford
Paulette Smith
Valerie Speights
Eugenia Spohrer
Amy Wilson